

**School of Public Health
Department of Epidemiology & Biostatistics**

**Antioxidant and omega-3 fatty acid intake in the modulation of
respiratory illness & asthma in children**

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Doctor of Public Health
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DECLARATION

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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ABSTRACT

Background and aim: Asthma is one of the major public health problems in Australia with prevalence in West Australian children reported up to 31%. The rise in asthma prevalence in Western societies may be related to changes in dietary habits, as diets are often low in antioxidant nutrients and omega-3 fatty acids (n-3) and high in omega-6 fatty acids (n-6).

The association between antioxidants, fatty acids and asthma is however controversial and there is a need for further investigation to clarify the role of these nutrients on asthma.

The aim of this study was to investigate the dietary exposures of n-3, n-6 and antioxidant nutrients proposed to have protective effects against asthma symptoms. Data gathered over fourteen years in a prospective pregnancy cohort study were analysed. We hypothesised that n-3 fatty acids and antioxidant nutrients (vitamins A, C, E, β -carotene antioxidants manganese and zinc) would be protective against asthma symptoms.

Methods: The Western Australian Pregnancy Cohort (Raine) study is a longitudinal study following 2868 children from birth to 14 years of age. This project consisted of two analyses as part of the overall study. Study One was a cross-sectional analysis of 1531 children at age 14 identified as having current asthma (n=167) or no asthma (n=1364). Study Two was a longitudinal analysis from a subset of the overall study whereby children were identified as having current asthma or no asthma at eight (n=335) and 14 (n=242) years of age. Dietary information was collected from food frequency questionnaires (FFQ) in both studies and red blood cell fatty acid content was measured in children for Study One. Student's t-tests and ANOVA allowed comparisons of food and nutrient intakes between children with asthma or no asthma for both studies. In Study One depending on the outcome of interest, multinomial or binary logistic regression was performed. In Study Two, food and nutrients that showed a significant difference between asthma groups in ANOVA analyses (p

<0.05) were included in analysis of covariance (ANCOVA) to determine whether they made an independent contribution after adjustment for age, sex, BMI and total energy intake. The Bonferroni post hoc test was applied to control for false positive results.

Results of Study One: The participants were 50.8% male (n= 778) and 49.2% female (n=753). One hundred and sixty seven adolescents (10.9%) had asthma categorised as mild (4.0%), moderate (4.4%) or severe (2.5%). Two hundred and eighteen adolescents (14.2%) had current wheeze. The most common allergy outcome was atopy (44.5%) n= 682. The least common allergy outcome was eczema (10.7%).

The large majority of dietary antioxidants and fatty acids tested were unrelated to asthma and asthmatic symptoms. However, after performing regression analysis and adjusting for potential confounders (BMI, age, gender and total daily energy intake) increased intake of vitamin C showed protective association with asthma (adjusted OR= 0.997, 95% CI= 0.994–0.999) and increased consumption of berry fruits showed protective effects on atopy (adjusted OR= 0.988, 95% CI= 0.976–0.999).

Increased level of docosahexaenoic acid (22:6n3) in RBC membrane showed protective effects on BHR (adjusted OR= 0.814, 95% CI= 0.669–0.989). The adjusted ratio of n6:n3 showed detrimental association with asthma (OR= 1.072, 95% CI= 1.009–1.139) and wheezing episodes (OR= 1.064, 95% CI= 1.007–1.124).

Results of Study Two: None of the antioxidant nutrients were shown to be associated with asthma. Unadjusted ANOVA analysis demonstrated a significant association between asthma diagnosis and arachidonic acid (20:4n6) (p=0.039). However, after performing ANCOVA and controlling for the confounders (age, sex, BMI and total energy) the association became non-significant (p=0.330). In crude analyses the n6:n3 ratio was higher for asthmatic adolescents at eight and 14 years (3.38) compared to those with no asthma (2.78) (p=0.013). This ratio remained significant after adjusting for the confounders (p=0.030)

Conclusions: Study One and Two provide some evidence on protective effects of antioxidants and fruits on asthmatic symptoms in adolescents. We found that while

controlling for potential confounders, increasing intake of vitamin C by 1 mg/day results in a 0.3% reduction in the risk of developing asthma. We demonstrated that for every 1 g/day increased intake of berry fruits there was 1.2% reduction in the risk of having atopy. We showed that by increasing 1 mmol/L in the level of docosahexaenoic acid in RBC membrane there was 18.6% reduction in the risk of having BHR. We also demonstrated that for every 1 unit increase in n6:n3 ratio, there was 6.4% increase in the risk of developing wheezing episodes and 7.2% increase in the risk of having asthma. Therefore, Our study suggests that the ratio of n6:n3 in diet may play a modulatory role in the expression of asthma, suggesting that promotion of increased total n-3 and reduced n-6 may protect against asthmatic traits in childhood and early adolescence.

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LIST OF ABBREVIATIONS

| <u>Acronym</u> | <u>Definition</u> |
|-----------------------|--|
| AA | Arachidonic acid |
| ALA | Alpha-linolenic acid |
| APC | Antigen-presenting cell |
| BHR | Bronchial hyperresponsiveness |
| CD | Cluster of differentiation |
| CI | Confidence interval |
| COX | Cyclo-oxygenase |
| DHA | Docosahexaenoic acid |
| DNA | Deoxyribonucleic acid |
| DPA | Docosapentaenoic acid |
| EPA | Eicosapentaenoic acid |
| FEV1 | Forced expiratory volume in 1 second |
| FFQ | Food frequency questionnaire |
| g/day | Grams per day |
| GSH | Glutathione peroxidase |
| Ig | Immunoglobulin |
| IL | Interleukin |
| ISAAC | International Study of Asthma and Allergies in Childhood |
| LA | Linoleic acid |
| mg | Milligram |
| mmol | Millimole |
| mRNA | Messenger ribonucleic acid |
| NK | Natural killer cell |
| OR | Odds ratio |
| PUFA | Polyunsaturated fatty acids |
| RBC | Red blood cell |
| RDI | Recommended daily intake |
| SD | Standard deviation |
| SE | Standard error |
| SEM | Standard Error of Mean |
| TNF | Tumour Necrosis Factor |

Introduction, objectives and hypotheses

The prevalence of asthma in children and young adults has increased over the last two decades in many Western countries including Australia. Asthma affects millions of Australians, including one in four primary school children and one in seven teenagers (Lincoln et al. 2006). Asthma is one of the major public health problems in Australia. In Perth, asthma has been diagnosed in 31% of children (Oddy et al. 2004a) which is even higher than the national prevalence of 25%. There is however emerging evidence suggesting that the prevalence of childhood asthma in Australia has reached a plateau (ACAM 2008).

The rise in asthma prevalence in Western societies may be related to changes in dietary habits. Despite the fact that there have been several studies on the impact of diet and its role in relation to asthma, the effect of diet on asthma remains unclear and requires further investigation.

Asthma is characterized by the chronic inflammation of the airways. Inflammatory cells generate large amounts of oxidants on the epithelial surfaces, leading to epithelial cellular injury (Patel et al. 2006). Antioxidants provide a defensive system in which the survival and development of cells in an oxidant-containing environment is possible (Hoffmann et al. 2007). Western diets are low in antioxidant nutrients which raises the possibility that a lack of these nutrients might explain the rising number of asthmatic children in Western countries (Devereux & Seaton 2005). Currently however, information on the relationship between asthma and antioxidants in the diets of children is scarce although some studies in adults have found modest beneficial effects (Kirkham & Rahman 2006; Troisi et al. 1995).

There is also increasing evidence that a diet with high levels of n-3 fatty acids has a protective effect against cough and wheeze both in children and adults (Castro-Rodriguez et al. 2008; Peat et al. 2004).

Consequently this project presents data gathered at 14 years in a prospective cohort study of 1799 adolescents of whom 1531 completed the food frequency questionnaires (FFQ). This study was designed to investigate the dietary exposures of fatty acids and antioxidant nutrients that have been proposed to reduce asthma symptoms.

This project consisted of two studies. Study One, using FFQ in Western Australian adolescents at 14 years investigated whether there was any association between the intake of antioxidants and n-3 fatty acids with asthma manifestations (wheeze, asthma, atopy and bronchial hyperresponsiveness). Also, for this study red blood cell fatty acid data were used to investigate the possible association of individual fatty acids, total n-3, total n-6 and the ratio of n6:n3 fatty acids on asthma.

In Study Two, two sets of data were used. The first used the FFQ when the children were eight years old (Oddy et al. 2004a) and the second when they were 14 years of age. These data were used for the analyses of the previously selected cases and controls at eight years of age and re-assessing their asthmatic and dietary status six years later when they were 14 years of age.

Therefore, the aim of Study One was to establish if there is an association between the intake of dietary factors of antioxidants and n-3 fatty acids and asthmatic traits (doctor diagnosed asthma, bronchial hyperresponsiveness (BHR), atopy, and episodes of wheezing) in adolescents. The aim of Study Two was to assess current asthmatic symptoms and dietary intake (fatty acid and antioxidant nutrients) of previously selected cases and controls and re-assess their asthmatic and dietary status six years later at 14 years of age through the follow up of participants who were assessed at eight years of age and had reached 14 years of age. Thus, Study Two allowed investigation of any possible causal associations of previous dietary exposures with current asthmatic symptoms.

Specific hypotheses of the study

The specific hypotheses of Study One were that:

1. consumption of specific antioxidants i.e. vitamins A, C, and E, β -carotene, zinc and fruits containing these nutrients will reduce asthmatic symptoms in 14 year old adolescents.
2. consumption of n-3 fatty acids reduces asthmatic symptoms in 14 year old adolescents.
3. higher level of n-3 fatty acids in red blood cells is associated with less asthmatic symptoms.

Study Two was hypothesised that:

1. consumption of specific antioxidants i.e. vitamins A, C, and E, β -carotene, zinc and fruits containing these nutrients is the lowest in adolescents with asthmatic symptoms at eight and 14 years compared to the non-asthmatic group and those with asthma at eight and 14 years only.
2. consumption of n-3 fatty acids is the lowest in adolescents with asthmatic symptoms at eight and 14 years compared to the group with no asthmatic symptoms and the groups who had asthma at eight and 14 years only.

CHAPTER ONE

CHARACTERISTICS OF ASTHMA

Asthma is a chronic illness involving the respiratory system in which the airway occasionally constricts, becomes inflamed, and is lined with excessive amounts of mucus, often in response to one or more triggers (Rodrigo & Nannini 2006). It is characterized by chronic inflammation of the airways. Inflammatory cells generate large amounts of oxidants on the epithelial surfaces, leading to epithelial cellular injury (Patel et al. 2006). The precise basis for the development of airway inflammation is not fully understood. One traditional theory postulates the development of an allergic response in the airways (Hoffmann et al. 2007).

1.1 DEVELOPMENT OF ASTHMA

The traditional inflammation theory in asthma development proposed that asthma is caused by increased recruitment of inflammatory cells from the bloodstream to the bronchial mucosa and also by enhanced survival of these cells in the inflamed airways (Pearce et al. 2007; Vignola et al. 2000). There are two cell death pathways by which an organism can discard cells that are superfluous or potentially harmful: necrosis and apoptosis (programmed cell death). Necrosis involves breakdown of the cellular membrane, which leads to leakage of intracellular proteins to the extracellular space and subsequently, inflammation (Figure 1). Necrosis usually affects large groups of cells while apoptosis typically involves single cells. In apoptosis cells undergo organised destruction of the cellular cytoskeleton and formation of apoptotic bodies, which are phagocytosed without an inflammatory reaction (Figure 1). Apoptosis is the most common form of physiological cell death and serves to eliminate cells that are potentially harmful to the body (Hoffmann et al. 2007; Simon 2003). Delayed apoptosis of inflammatory cells in inflammatory responses contributes to their accumulation in order to efficiently eliminate the cause of inflammation (Simon 2003). The survival of inflammatory cells in airway tissues depends on several factors including lack of antioxidants and the prolonged activity of reactive oxygen species (Simon 2003; Tripathi et al. 2007). Increasing amount of

evidence lends support to the concept that a dysregulation of cell apoptosis may play a central role in the development of airway inflammatory associated with asthma (Jeon et al. 2007; Radinger et al. 2007; Stern et al. 2007).

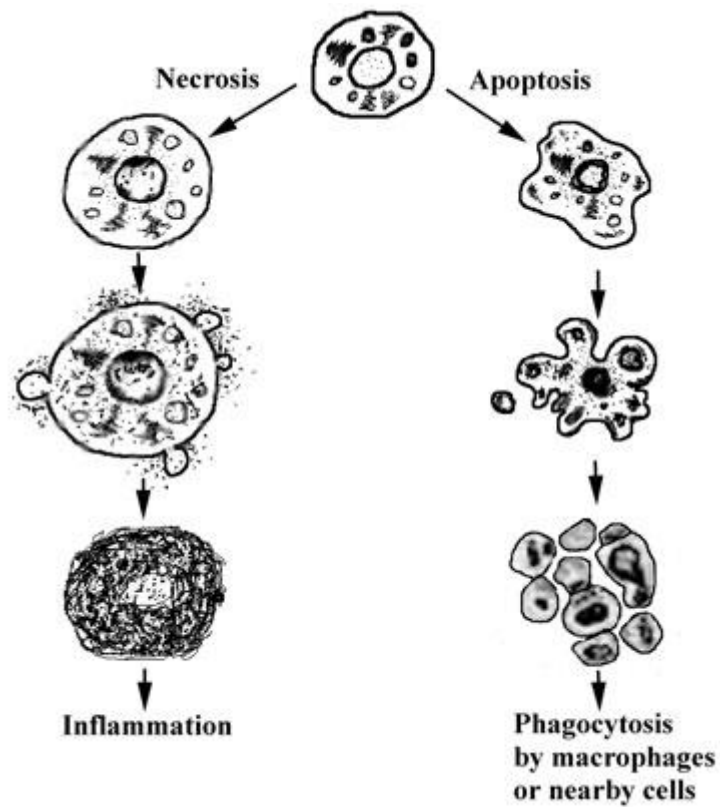


Figure 1 - Cell death pathway necrosis and apoptosis
(Simon 2003)

1.2 PHENOTYPES OF ASTHMA

Asthma phenotypes are differentiated based upon the development of symptoms and the severity of asthmatic lung inflammation (Janeway et al. 2001). Asthma symptoms are typically manifested at certain stages in life and can be classified into three general categories: childhood asthma, late-onset asthma and occupational asthma. Childhood asthma can arise from several different factors (Hang et al. 2003). Typically, a co-viral infection such as the rhinovirus, a family history of allergy, or atopy (an allergic hypersensitivity) can result in the development of childhood asthma (Pearce et al. 2007; Vignola et al. 2000). In childhood asthma, atopy usually results from innocuous substances such as dust mites, pet dander, and fungi (Hang et al. 2003). Late onset asthma (which starts in the middle or later in life) and occupational asthma exhibit different characteristics from childhood asthma and probably have a different aetiology (Pearce et al. 2007). Asthma's causation in these circumstances may arise from constant exposure to environmentally innocuous antigens. The current distinction between late-onset asthma and occupational asthma occurs because the latter happens usually because of specific antigen exposure related to work, while only 10 to 15 per cent of cases of late-onset asthma are work-related and the majority are triggered by viral infections (Pearce et al. 2007; Vignola et al. 2000).

Asthmatic inflammation is differentiated into three broad categories: acute, subacute and chronic. Acute asthmatic inflammation involves the early recruitment of cells into the airway, while subacute asthmatic inflammation is characterized by the activation of recruited and residual effector cells resulting in incessant inflammation (Hang et al. 2003). Chronic asthma is defined by constant inflammation leading to cellular damage, which in turn activates cellular repair (Hang et al. 2003).

1.3 ASTHMA SYMPTOMS AND MANIFESTATIONS

Common asthma symptoms include: coughing, wheezing, shortness of breath, chest tightness, pain, or pressure (Janssens et al. 2009). People with asthma experience symptoms when the airways tighten, are inflamed, or fill with mucus. Not every person with asthma has the same symptoms in the same way. Symptoms may vary from one asthma attack to the next, being mild during one and severe during another (Chen et al. 2006; Ritz et al. 2001).

Bronchial hyperresponsiveness (BHR), atopy and wheeze are the manifestations associated with asthma (Cornejo-Garcia et al. 2007; Floistrup et al. 2006; Marks et al. 2006; Stern et al. 2007; Sumi et al. 2007). These asthma manifestations are explored in more detail in the following sections.

1.3.1 Bronchial hyperresponsiveness (BHR)

Bronchial hyperresponsiveness (BHR) is defined as an increase in sensitivity to a wide variety of airway narrowing stimuli. In asthma, this hypersensitivity is accompanied by excessive degrees of airway narrowing (Sumi et al. 2007). It is currently believed that both muscular and non-muscular components of the airway wall are important determinants of the degree of airway narrowing that result from smooth muscle stimulation (Southam et al. 2007). Non-muscular components of the airway and the lung parenchyma can affect the degree of smooth muscle shortening and airway narrowing that occurs in response to airway smooth muscle stimulation (Southam et al. 2007; Weissman 2002).

1.3.2 Wheeze

Wheezing is a high-pitched sound associated with laboured breathing. Wheezing occurs when one tries to breathe deeply through air passages that are narrowed or filled with mucus (Southam et al. 2007; Varraso et al. 2007). Wheezing is also very closely associated with respiratory syncytial virus (RSV) (Anderson et al. 2005;

Schauer et al. 2002). Wheezing is commonly experienced by persons with a lung disease; the most common cause of recurrent wheezing is asthma, a form of reactive airway disease (Orient 2000; Pearce et al. 2007). In previous studies it has been documented that wheezing in the previous 12 months is positively correlated with atopy (Weissman 2002). Therefore, wheezing is the symptom most associated with asthma (Cornejo-Garcia et al. 2007).

1.3.3 Atopy

Atopy is an allergic hypersensitivity affecting parts of the body not in direct contact with the allergen. It may involve eczema (atopic dermatitis), allergic rhinitis and asthma. There appears to be a strong hereditary component (Floistrup et al. 2006; Socha et al. 2007). Although atopy has various definitions, most consistently it is defined by the presence of elevated levels of total and allergen-specific IgE in the serum of patients, leading to positive skin-prick tests to common allergens (Floistrup et al. 2006; Stern et al. 2007).

Among asthmatic patients reporting wheezing in the previous 12 months, a stronger relationship has been noted with atopy for those reporting more than 12 episodes of wheezing than for those reporting 1 to 3 episodes ([OR] 8.70 vs. 3.27, respectively) (Marks et al. 2006). Atopy has also been found to be more strongly associated with hospital attendance for asthma than with ever having had asthma (OR, 16.95 vs. 2.09, respectively) (Marks et al. 2006). The proportion of “asthma-ever” attributable to atopy is reported as 33% in one study (Weissman 2002), while for hospital attendance in 1999, this proportion was 89% (Marks et al. 2006). Based on these findings, it is suggested that atopy may contribute more to frequent or severe asthma than to infrequent or mild asthma (Weissman 2002).

1.4 IMMUNOLOGICAL RESPONSES IN ASTHMA

The immune system is divided into the innate immune system, and the acquired or adaptive immune system. The latter is further divided into humoral and cellular components (Berrington et al. 2005). Humoral immunity is the aspect of immunity that is mediated by secreted antibodies, produced by B lymphocytes. Secreted antibodies bind to antigens on the surfaces of invading micro-organisms which flags them for destruction (Tripathi et al. 2007).

Cellular immunity on the other hand, is an immune response that does not involve antibodies but rather involves the activation of macrophages, natural killer (NK) cells, and the release of various cytokines in response to an antigen. This type of response is what is typically observed in asthma. Cellular immunity protects the body by activating antigen-specific cytotoxic T-lymphocytes that are able to induce apoptosis (programmed cell death or cellular suicide). Cellular immunity also protects the body by stimulating cells to secrete a variety of cytokines that influence the function of other cells involved in adaptive immune responses and innate immune responses.

Asthmatic symptoms arise from the activation of sub-mucosal mast cells by innocuous antigens (allergens) in the lower airways resulting in mucous and fluid accumulation subsequently followed by bronchial constriction (Radinger et al. 2007). The immune response to asthmatic allergens is mediated by CD4⁺ T helper 2 (T_H2) cells, eosinophils, neutrophils, macrophages, and IgE antibodies (Jeon et al. 2007; Radinger et al. 2007; Stern et al. 2007) (Figure 2).

1.4.1 Lymphocytes and lymphoid cells

Lymphocytes are a type of white blood cell (leukocytes) in the immune system. Lymphoid cells are considered to be the precursor for the mature lymphocytes. An average human body contains about 10¹² lymphoid cells (Radinger et al. 2007). The

lymphoid tissue as a whole represents about 2% of the total body weight (Berrington et al. 2005).

The three major types of lymphocyte are T cells, B cells and Natural Killer (NK) cells (Stern et al. 2007). The small lymphocytes are the T cells and B cells. Lymphocytes play an important and integral role in the body's defences.

T cells and B-cells are the major cellular components of the adaptive immune response (Tripathi et al. 2007). T cells are involved in cell-mediated immunity whereas B cells are primarily responsible for humoral immunity. The function of T cells and B cells is to recognize specific non-self antigens, during a process known as antigen presentation. Once they have identified an invader, the cells generate specific responses that are tailored to maximally eliminate specific pathogens or pathogen infected cells (Tripathi et al. 2007). B cells respond to pathogens by producing large quantities of antibodies which then neutralize foreign objects like bacteria and viruses (Radinger et al. 2007).

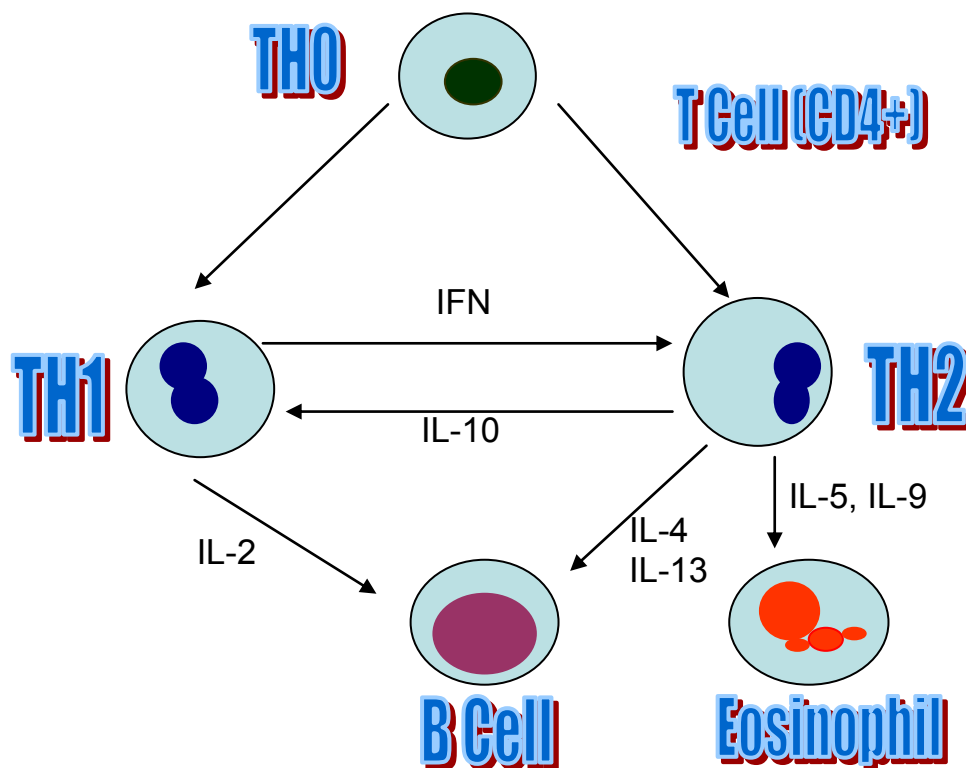


Figure 2-Development of systemic allergy (including asthma)
(Compiled by the candidate)

In response to pathogens some T cells, called helper T cells produce cytokines that direct the immune response whilst other T cells, called cytotoxic T cells, produce toxic granules that induce the death of pathogen infected cells. In general, allergens induce a CD4 T helper (T_H) cell response, whereas viruses recognize CD8+ cytotoxic T cells (T_c). In the asthmatic airways, there are CD4+ and, to a lesser number CD8+ cells with a type 2 cytokine phenotype (i.e., T_H -2 and T_c -2 type) (Jeon et al. 2007). These cells produce interleukin (IL) 3 and 5 and granulocyte-macrophage colony-stimulating factor which recruit, mobilize and activate eosinophils for subsequent mucosal damage, as well as IL-4, an essential cofactor for local or generalized IgE production (Stern et al. 2007) (Figure 2). The activation of eosinophils also leads to epithelial shedding, mucus hypersecretion and bronchial muscle contraction. Thus, although the eosinophil may damage the mucosal surfaces in asthma, its function appears to be under T cell control (Jeon et al. 2007; Radinger et al. 2007). Eosinophilic inflammation represents an essential element in the pathogenesis of asthma (Hasala et al. 2008).

1.4.1.1 T-lymphocytes

Helper T cells have two important functions: to stimulate cellular immunity and inflammation and to stimulate B cells to produce antibody. CD4+ helper T cells are capable of differentiating from an initial common state (T_{H0}) into two apparently distinct types called T_{H1} and T_{H2} . These subtypes differ in their cytokine secretion (Radinger et al. 2007).

The balance between T_{H1} and T_{H2} represents a switch mechanism which can be used to bias the immune response in one direction or another. The commitment of T_{H0} cells to become T_{H1} or T_{H2} is influenced by cytokines secreted by the two subtypes themselves and by macrophages. The outcome of the differentiation switch between different subtypes of T_H cells is activation of the two different pathways of immunity which are associated with different antibody isotypes (Berrington et al. 2005).

The T_{H1} pathway is essentially cell mediated immunity, with the activation of macrophages, NK cells, cytotoxic T cells and a prolonged inflammatory response.

The cytokines secreted by T_H1 cells also boost production of IgG₂ antibody production in mice (Radinger et al. 2007). T_H1 cells produce IL-2, IFN γ , and TNF β , which activate macrophages to stimulate cellular immunity and inflammation. T_H1 cells also secrete IL-3 and Granulocyte Monocyte Colony-Stimulating Factor (GM-CSF) to stimulate the bone marrow to produce more leukocytes.

The T_H2 pathway is essentially a humoral pathway, with the production of cytokines which promote B cell growth (like IL-4, IL-6) and production of IgG (IL-4), IgA (IL-5) and IgE (IL-4) in mice. T_H2 also stimulates effectors which use these antibody isotypes; eosinophils (via IL-5) and mast cells (IL-4) (Radinger et al. 2007; Tripathi et al. 2007).

The balance between T_H1 and T_H2 activity may steer the immune response in the direction of cell-mediated or humoral immunity (Figure 2) (Cornejo-Garcia et al. 2007).

T lymphocytes play a central role in the development of airway inflammation. They are present in increased numbers in the airways of patients with fatal asthma or in patients with asthma of variable aetiology including occupational asthma (Sumi et al. 2007; Tripathi et al. 2007). It has been reported that there is an inverse association between childhood infectious illness and the development of atopy, suggesting that certain forms of infection protect against asthma. This may involve a shift in the balance of CD4⁺ T- lymphocyte helper cells from a T_H2 to a T_H1-type cytokine profile (Mawson 2001).

1.4.1.2 B-lymphocytes

B-lymphocytes play a vital role in the body's specific immune system. These cells are not only produced in the bone marrow, but also mature there. B-cells mature into plasma cells and are responsible for the production of antibodies, which primarily play a role in bacterial infections (Sumi et al. 2007). In asthma, B cells can play an important role as an antigen-presenting cell (APC) in the development of primary CD4⁺ T cell responses (Cornejo-Garcia et al. 2007).

1.4.2 Cytokines and eosinophils

Cytokines are small secreted proteins (between 8 and 30 kilo Daltons or kDa) which mediate and regulate immunity, inflammation, and hematopoiesis (Jeon et al. 2007). They mostly act over short distances and short time spans and at very low concentration. They act by binding to specific membrane receptors, which then signal the cell via second messengers, often tyrosine kinases, to alter its behaviour (gene expression) (Cornejo-Garcia et al. 2007) (Figure 3).

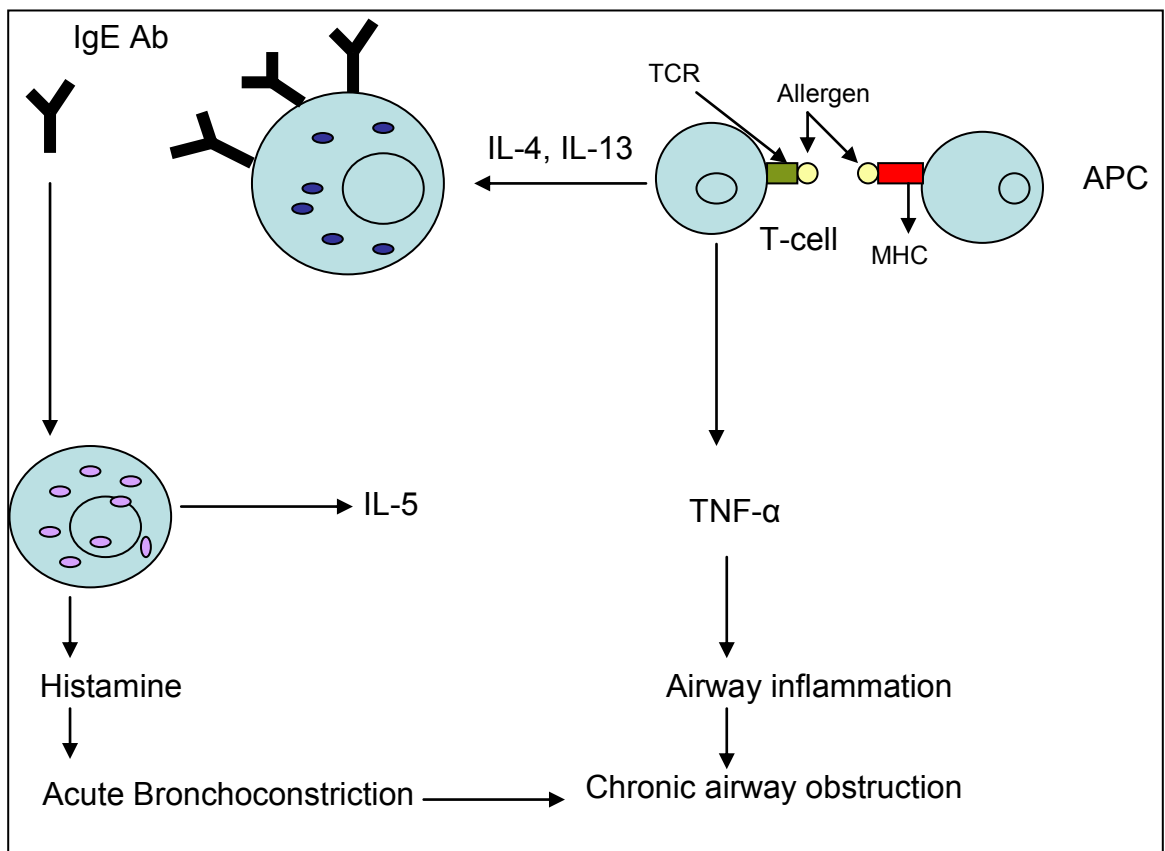


Figure 3- Schematic diagram of the role of the inflammatory cells in asthma
(Hasala et al. 2008; Knutsen et al. 2008; Larocca et al. 2008)

Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules.

The largest group of cytokines stimulates immune cell proliferation and differentiation. This group includes Interleukin 1 (IL-1), which activates T cells; IL-

2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5, and IL-6, which stimulate proliferation and differentiation of B cells; Interferon gamma (IFN γ), which activates macrophages; and IL-3, IL-7 and GM-CSF, which stimulate hematopoiesis (Radinger et al. 2007).

Other groups of cytokines include interferons and chemokines (Cornejo-Garcia et al. 2007). Interferons IFN α and IFN β inhibit virus replication in infected cells, while IFN γ also stimulates antigen-presenting cell MHC expression. Cytokines act on their target cells by binding specific membrane receptors. The receptors and their corresponding cytokines have been divided into several families based on their structure and activities (Cornejo-Garcia et al. 2007).

In asthmatics eosinophils release a variety of pro-inflammatory cytokines including IL-3 and IL-5. They also release highly toxic granule-derived basic proteins (such as eosinophil cationic protein or ECP) that are responsible for epithelial cell damage in chronic asthma (Jeon et al. 2007).

The balance between eosinophil maturation, recruitment and removal largely determines the number of eosinophils in the blood and tissues (Bratke et al. 2007; Hasala et al. 2008). As discussed earlier apoptosis, or programmed cell death, is a controlled process of cell suicide that allows the removal of aging cells or those whose continued survival would be detrimental for the organism (Hasala et al. 2008; Knutsen et al. 2008; Radinger et al. 2007). Furthermore, failure in the apoptotic process of eosinophils has been associated with pulmonary and allergic diseases (Hasala et al. 2008). NK cells are a part of the innate immune system and play a major role in defending the host from both tumours and virally infected cells by recognizing alterations in levels of a surface molecule called Major Histocompatibility Complex (MHC). NK cells are activated in response to interferons. Activated NK cells release cytotoxic granules which then destroy the altered cells.

1.4.3 Histocompatibility molecules

Histocompatibility molecules are glycoproteins expressed at the surface of almost all vertebrate cells. They get their name because they are responsible for the compatibility (or the lack of it) of the tissues of genetically different individuals (Bratke et al. 2007). Only monozygotic (identical) human twins have the same histocompatibility molecules on their cells, and therefore, they can accept transplants of tissue from each other. The rest of us have a set of histocompatibility molecules that is probably unique to us. A graft of our tissue into another human will provoke an immune response which, if left unchecked, will end in the rejection of the transplant (Bratke et al. 2007). So the histocompatibility molecules of one individual act as antigens when introduced into a different individual and are also called histocompatibility antigens.

There are two categories of histocompatibility molecules: class I and class II. Class I molecules serve to display antigens on the surface of the cells so that they can be recognized by T cells. These molecules provide tissue identity and serve as major targets in the rejection of transplanted tissue (Bratke et al. 2007; Cornejo-Garcia et al. 2007). Three different types of class I molecules are: human leukocyte antigen A, B and C designated as HLA-A, HLA-B, and HLA-C. Class II molecules are designated HLA-D (Radinger et al. 2007). Class II molecules, in contrast to class I, are normally expressed on only certain types of cells. These are cells like macrophages and B lymphocytes that specialize in processing and presenting extra cellular antigens to T- lymphocytes (Cornejo-Garcia et al. 2007; Radinger et al. 2007). Most of the T cells of the body belong to one of two distinct subsets: cluster of differentiation 4 ($CD4^+$) or cluster of differentiation 8 ($CD8^+$). $CD4^+$ and $CD8^+$ are surface glycoproteins (Radinger et al. 2007). The CD8 molecules on $CD8^+$ T cells bind to a site found only on class I histocompatibility molecules while $CD4^+$ molecules on $CD4^+$ T cells bind to a site found only on class II histocompatibility molecules (Radinger et al. 2007). In asthma most cells bear $CD4^+$ receptors while $CD8^+$ cells are rarely identified (Sumi et al. 2007).

1.4.4 The allergic response and development of the asthma phenotype

Antigen is inhaled into the airway and is taken up and processed by an antigen-presenting cell (APC) (Hoffmann et al. 2007). The APC then migrates from the airway mucosa to the regional lymph nodes. Upon arrival at the lymph node, the APC cell, which has matured along the journey, presents the processed antigenic peptides to T cells. Rare antigen-specific T cells sample the peptides being presented in the lymph node; when an antigen-specific T cell finds an APC cell that presents the peptides for which the T cell is specific, it begins to proliferate (Flostrup et al. 2006; Hoffmann et al. 2007). Simultaneously, the APC, through various signals, attempts to skew the cytokine profile of the developing T cells to cause them to become T helper type 1 (T_H1) cells (which primarily produce interferon gamma [IFN- γ]) and interleukin 12 [IL-12]) or T_H2 cells (which produce IL-4, IL-5, and IL-13) (Galli & Tsai 2007; Hoffmann et al. 2007). These cytokine profiles may be found in both CD4⁺ and CD8⁺ T cells; both types of T cells have been implicated in causing asthma (Galli & Tsai 2007).

The allergic response in general and the asthmatic allergic response in particular depend on a T_H2 response to an antigen. T_H2 cells produce IL-4, which causes B cells to switch from usual production of IgM or IgG antibody to production of IgE antibody (Jeon et al. 2007). Once produced, IgE antibodies bind to the surface of mast cells and basophils, where subsequent antigen cross-linking causes the generation and release of mediators that drive the asthma phenotype (Galli & Tsai 2007).

In an experimental model of the allergic response to inhaled antigen, T_H2 cells alone were not sufficient to cause the asthma phenotype, because T_H2 cell recruitment to the allergic site depends on help from T_H1 cells (Jeon et al. 2007). T_H1 cells are more readily recruited to the airway tissue and are responsible for tumour necrosis factor (TNF) dependent expression of vascular cell adhesion molecules; the presence of T_H1 cells in the airway tissue in turn allows T_H2 cells to enter the tissue (Jeon et al. 2007; Stern et al. 2007). The T_H1 cells do not need to be of the same antigenic specificity as the T_H2 cells. This finding may help explain why inflammatory stimuli,

such as respiratory viral infections, may facilitate the allergic response and contribute to flares of allergic asthma.

Allergens may also be sufficient to produce airway inflammation. When a sensitized individual is re-exposed to an allergen, the subsequent cross-linking of IgE on the surface of the mast cells and basophils can lead to the release of immediate-phase reactants such as histamine and TNF (Jeon et al. 2007). Within 4 to 6 hours after exposure, the activated immune cells produce chemokines and cytokines, proteinases, enzymes, and lipid mediators such as the cysteinyl leukotrienes (Radinger et al. 2007). Chemokines recruit and help activate additional T_H2 cells and eosinophils in the airway. Proteinases and other enzymes may lead to airway damage, which promotes collagen deposition in the subepithelial basement membrane region. Cysteinyl leukotrienes can cause airway smooth muscle constriction and mucous cell secretion that further obstruct the airway lumen (Duramad, Tager & Holland 2007).

Leukotrienes in concert with chemokines (e.g., eotaxin) and cytokines (e.g., IL-5) may also recruit and activate eosinophils in the airway. Eosinophils are granulocytes that have a very short life span in the periphery and do not appear in large amounts in the circulation. These cells are produced in the bone marrow under the influence of T_H2 cell production of IL-5. Newly produced eosinophils are released into the circulation and settle in the lung tissue, guided by a similar set of cell adhesion molecules that direct T_H2 cell traffic. Upon entry into airway tissue, eosinophils and T_H2 cells secrete their products, which, in addition to the mast cell/basophil products, are thought to lead to mucous cell metaplasia and smooth muscle hyperplasia (Duramad, Tager & Holland 2007). These changes in cellular behaviour are the basis for the hypersecretion and hyperresponsiveness that is characteristic of the asthma phenotype. The T_H2 cytokines IL-9 and IL-13 are especially effective in driving differentiation of mucous cells. Eosinophil granule constituents such as myelin basic protein, eosinophilic cationic protein, and eosinophil-derived neurotoxin may also contribute to airway inflammation, damage, and re-modelling, although recent findings in mouse models and human subjects suggest that the contribution of the eosinophil may not be necessary for the asthma phenotype, at least under some conditions (Haldar & Pavord 2007). Recently, an additional type of T cell, the

regulatory T cell (Tr) also known as suppressor T cell, was identified (Kapp & Bucy 2008). Tr cells produce IL-10 and transforming growth factor- β (TGF- β), which may down regulate the immune response. Studies in experimental models suggest that an inhibition of the Tr function may also drive the asthma phenotype, but its role in humans is not yet clear (Duramad, Tager & Holland 2007).

Nutritional deficiency is frequently associated with impaired immune responses, mostly cell-mediated immunity, including cytokine production, secretory antibody responses and the complement system (Calder 2006; Chandra 1991). Mazari & Lesourd (Mazari & Lesourd 1998) compared the influences of aging and nutrition on immune responses of healthy elderly (80 ± 5 years) with different nutritional status to young healthy adults (25 ± 5 years). They reported that the influences of aging and undernutrition are cumulative and that some changes in immune response that have been attributed to aging may, in fact, be related to nutrition and not aging. For instance, minor changes in nutritional status, such as lower folate levels, were associated with lower CD_4^+ subsets in peripheral blood in healthy young adults as well as their healthy elderly counterparts. The associations between nutrition and the development of asthma will be further discussed in section 1.7.

1.5 ATOPIC AND NON-ATOPIC ASTHMA

Asthma that results from sensitivity to specific external allergens is known as atopic (extrinsic) asthma (Hasala et al. 2008). In cases in which the allergen isn't obvious, asthma is referred to as non-atopic (intrinsic) asthma (Hasala et al. 2008). Allergens that cause atopic asthma include pollen, house dust or mould, feather pillows, food additives containing sulphites, and any other sensitizing substance (Wichmann et al. 2008). Atopic asthma usually begins in childhood and is accompanied by other manifestations of atopy such as eczema and allergic rhinitis (Knutsen et al. 2008). In non-atopic asthma, no extrinsic allergen can be identified. Most cases of non-atopic asthma are preceded by a severe respiratory infection. Irritants, emotional stress, fatigue, exposure to noxious fumes as well as changes in endocrine, temperature, and humidity may aggravate non-atopic asthma attacks (Moreira et al. 2008; Varraso et

al. 2007; Wichmann et al. 2008). In many asthmatics, both forms of intrinsic and extrinsic asthma coexist.

The expression of messenger ribonucleic acid encoding IL-13 (IL-13) mRNA in the bronchial mucosa of both atopic and non-atopic subjects is shown to be significantly higher compared with non-asthmatics ($p \leq 0.02$) (Chanez et al. 2007). IL-13 cytokines are involved in airway hyperresponsiveness, which as discussed earlier, is a critical event in patients with asthma (Knutsen et al. 2008; Larocca et al. 2008). Thus, it is not surprising to observe more BHR positive reactions in both atopic and non-atopic asthmatics as opposed to non-asthmatic individuals due to a higher level of IL-13 in them.

There is increasing evidence that inflammatory mechanisms other than eosinophilic inflammation may be involved in producing the enhanced bronchial reactivity and reversible airflow obstruction that characterises asthma (Halder and Pavord 2007). At most, only 50% of asthma cases are attributable to eosinophilic airway inflammation. It is hypothesised that a major proportion of asthma is based on non-eosinophilic (neutrophilic) airway inflammation, possibly triggered by environmental exposure to bacterial endotoxin, air pollution and viral infections (Halder and Pavord 2007). Distinguishing between the two subtypes of asthma (eosinophilic and non-eosinophilic), could have major consequences for the treatment and prevention of asthma. Non-eosinophilic asthma represents a stable phenotype associated with a distinct lower airway pathology and structure. Eosinophilic asthma is considered a consequence of allergen-mediated activation of mast cells and T cells in the airway with release of TH₂ cytokines. In contrast, non-eosinophilic asthma is the product of innate and cell mediated immune responses.

A Western diet (low antioxidant and high saturated fat intake) may activate innate immune responses in asthma by initiating an NF κ B-mediated inflammatory cascade (Wood and Gibson 2009). A high fat diet results in production of pro-inflammatory mediators such as IL-6, IL-8, TNF α and may lead to activation of neutrophils. Low antioxidant intake reduces the host's ability to scavenge reactive oxygen species produced in response to triggers such as viruses and endotoxin. This will exaggerate the inflammatory response by further activation of NF κ B (Wood and Gibson 2009).

1.6 ASTHMA RISK FACTORS

Asthma, like many other allergic reactions can be associated by a variety of different risk factors. Many of these factors, as we shall see are closely related. For instance low socioeconomic status is closely related with low income and the latter is usually closely related with lower level of education. This will be discussed in more detail in future sections (1.6.1-1.6.3).

1.6.1 Socioeconomic status

Socioeconomic status covers a wide range of living aspects of every human being. It determines the location where a person can afford to live; this in turn could determine the level of exposure to environmental pollutions. For instance, although asthma is only slightly more prevalent in the United States among minority children than among whites, it accounts for three times the number of deaths (Moreira et al. 2008).

Poverty, substandard housing that increases exposure to certain indoor allergens, lack of education, inadequate access to health care, and the failure to take appropriate prescribed medications may all increase the risk of having a severe asthma attack or, more tragically, of dying from asthma (Moreira et al. 2008; Pearce et al. 2007; Wichmann et al. 2008). Poverty contributes to disease in numerous ways. It reduces people's access to health care, reduces treatment availability, lowers overall educational levels, and subjects people to reduced standards of housing and related utilities (Moreira et al. 2008; Pearce et al. 2007; Wichmann et al. 2008). Poverty is a substantial health risk even in developed countries, but is particularly serious in undeveloped countries (Pearce et al. 2007).

In a recent study, a significant positive association between income and asthma stressed the importance of improvement in economic situations as a means for preventing asthma occurrence and improving quality of life (Moreira et al. 2008).

1.6.2 Family history

Family history of asthma in one or more first-degree relatives has been consistently identified as a risk factor for asthma in several studies (Burke et al. 2003; Pole et al. 2008; Scirica et al. 2007; Socha et al. 2007; Varraso et al. 2007). In one study it was shown that having a parent with a history of asthma doubles the risk of asthma for their children (OR 2.05, 95% CI 1.34 to 3.16) (Haby et al. 2001).

In another study, investigators (Burke et al. 2003) identified 33 studies from all geographic regions of the world for review. The asthma prevalence ranged from 2% to 26% (Burke et al. 2003). Positive predictive value of family history was calculated as 11% to 37%, and negative predictive value from 86% to 97% (Burke et al. 2003). Therefore, for a person with no family history of asthma it could be more reliably predicted that this person would not become asthmatic compared with a person who had a positive family history of asthma.

Hundreds of gene linkage analyses studies have identified several chromosomal regions which harbour asthma susceptibility genes like chromosomes 2q, 5q, 6q, 11q, 12q and 13q (Bierbaum & Heinzmann 2007). However, studies performed in farmers' children have shown that exposure to bacterial endotoxin early in life reduces the risk to develop asthma or atopy later on life (Koppelman et al. 2002). Therefore, this demonstrates the complexity of asthma incidence and highlights the fact that genetic predisposition is not the only factor that plays a crucial role in the incidence of asthma as environment factors may also play a significant role.

1.6.3 Virus infection

Children with recurrent virus-induced wheezing episodes are at great risk for chronic childhood asthma (Gern et al. 2005). Infancy is a time of increased susceptibility to viral infections, and a time for extensive re-modelling of the airways to accommodate growth. The observation that children with asthma can have structural lung changes and functional deficits at an early age, suggests that viral infections could adversely affect lung development. Infections with respiratory viruses can acutely impair lung function by directly damaging lower airway tissues and by provoking an acute immune response with both antiviral and pro-inflammatory properties (Gern et al. 2005). Virus induced epithelial damage can also increase the permeability of the mucosal layer and facilitates allergen contact with immune cells to promote inflammation. Viruses initiate inflammatory and antiviral responses by binding to specific receptors on the surface of cells, activating intracellular signalling pathways, and generating oxidative stress. These events lead to the activation of innate antiviral pathways, and the release of a variety of cytokines. As a result, neutrophils and mononuclear cells are recruited to the area of infection and are in turn activated to secrete pro-inflammatory cytokines, such as IL-1, IL-8, IL-10 and TNF- α . Changes in IL-8 levels in nasal secretions have been related to virus-induced increases in airway hyperresponsiveness suggesting that neutrophil activation products contribute to airway obstruction and symptoms during viral infections and asthma. Animal experiments confirm the concept that viral infections may have to occur in a genetically susceptible host at a critical period in the development of the immune system for asthma inception to occur in early childhood (Wood and Gibson 2009).

1.6.4 Diet

Diet is another factor proposed to play a role in the incidence of allergic manifestations including asthma and is the main focus of this research. In a recent study conducted on Japanese female university students (n=153, 32.7% asthmatics, 25% with wheezing episodes), meat consumption was related to wheeze (OR=2.00; 95% CI 1.12–3.60) and respiratory infections (OR=2.10; 95% CI 1.08–4.09)

(Takaoka & Norback 2008). Fish consumption was related to less respiratory infections (OR=0.49; 95% CI 0.28–0.86), and milk consumption to less daytime breathlessness (OR=0.72; 95% CI 0.55–0.95). Fast food consumption was also related to wheeze (OR=1.89; 95% CI 1.23–2.91 and daytime breathlessness (OR=1.50; 95% CI 1.00–2.28) (Takaoka & Norback 2008). Those university students consuming butter (OR=2.65; 95% CI 1.11–6.32) and rapeseed oil (OR=2.35; 95% CI 1.03–5.38) had more wheeze. Those consuming margarine had more breathlessness (OR=4.40; 95% CI 1.42–13.70) (Takaoka & Norback 2008). An asthma symptom score was related to fast food ($p<0.05$) and margarine consumption ($p<0.01$). It was concluded that fish, seafood and milk consumption were beneficial, while butter, margarine, rapeseed oil, fast food and soft drinks could be risk factors for allergy and poor respiratory health.

Diet during infancy is also a significant factor in the development of asthma. The introduction of milk other than breast milk to infants before four months of age is shown to be a significant risk factor for all asthma and atopy related outcomes in children later on at six years of age (Bush 2009; Hanson, Korotkova & Telemo 2003; Oddy et al. 1999; Peroni, Chatzimichail & Boner 2002)

It has also been recently shown that the Mediterranean diet which is rich in fruits and vegetables, nuts, grains, olive oil (as opposed to butter) and grilled or steamed chicken and seafood (as opposed to red meat), is an independent protective factor for current wheezing in preschoolers, irrespective of obesity and physical activity (Castro-Rodriguez et al. 2008).

1.7 NUTRITION AND ASTHMA

As discussed above, nutrition and diet can play an important role in the development of asthma. In this chapter the nutrients potentially implicated in the development of asthma and their proposed mechanism of action are discussed in detail.

The incidence of asthma in children and young adults has increased over the last two decades in many countries including Australia. However, this trend is not the same

among all countries (Patel et al. 2006). In fact there is, greater than 15-fold difference in incidence between countries worldwide. The prevalence of asthma is also different between countries. It is low in Eastern Europe and Asia for instance but much higher in the United Kingdom, Australia, New Zealand, Ireland, and America (Tabak et al. 2005). Diet is one of several causal factors implicated in the substantial increase in incidence of childhood asthma over the last two decades and the evidence based on the relation between diet and asthma has increased dramatically (McKeever & Britton 2004). Therefore, the rise in asthma incidence in Western societies may be related to changes in dietary habits. Emerging literature is now relating diet with asthma prevalence, an area of research which requires more in depth investigation (Bolte et al. 2006; Devereux & Seaton 2005; Kuiper et al. 2006; Patel et al. 2006; Tabak et al. 2005).

Several epidemiological studies have investigated diet as a potential risk factor for asthma, although the results from these studies have been inconclusive (Wiesch, Meyers & Bleecker 1999). Dietary fatty acid intake for example is proposed to contribute to asthma development with n-6 polyunsaturated fatty acids (PUFA) having detrimental effects and n-3 PUFA, protective effects (Bolte et al. 2006; Hodge et al. 1996). Frequent consumption of products containing milk fat in pre-school children has also been associated with a reduced risk of asthma symptoms (Wijga et al. 2003). In one study on teenagers, asthma has been positively associated with protein-rich and fat-rich foods of animal origin (Huang, Lin & Pan 2001).

Increased amount of saturated fatty acids intake has been associated with asthma risk in schoolchildren (eight to 13 years of age) in one study (Rodriguez et al. 2010). There are changes in the hormones derived from fat tissue that may affect the airways (Kattan et al. 2010). One of these hormones, leptin, is pro-inflammatory and obese individuals have higher leptin levels than lean individuals (Johnson et al. 2007). Leptin is produced by adipocytes and is thought to act primarily through specific receptors at the hypothalamus (Guler et al. 2004). Leptin receptors also exist in lung tissue, and leptin may have stimulatory effects on the proliferation of cells of a human cell line through its specific leptin receptor (Litonjua and Gold 2008). It may provide a link between inflammation and T-cell function in asthma. In animal models leptin up-regulates production of TNF- α , IL-6, and IL-12 (Guler et al. 2004).

It is found at higher levels among asthmatics regardless of the extent of obesity (Kattan et al. 2010). Leptin level has been suggested as being a predictive factor for having asthma in children (OR= 1.98; 95% CI, 1.10-3.55; $P = 0.021$) (Guler et al. 2004).

In contrast, blood levels of another hormone; adiponectin (an insulin sensitizing hormone) has anti-inflammatory properties and correlates inversely with obesity (Shore 2008). Adiponectin acts on macrophages and monocytes to inhibit production of pro-inflammatory cytokines and to augment IL-10 and IL-1 receptor antagonist expression (Shore 2008).

Therefore there are many studies which suggest that dietary factors have an important role in the worldwide increases in asthma. There are however, other studies, which do not suggest a role for diet in the incidence and manifestation of asthma. In a recent study, there were no significant differences between the serum antioxidant levels in the asthmatic group compared with the control group (Emecen et al. 2009). Another study found that dietary intake of n-3 and n-6 fatty acids are not associated with any respiratory or allergic outcomes (Almqvist et al. 2007).

1.7.1 Dietary antioxidants and the prevalence of asthma

Antioxidants are defined by their mechanism of action, i.e. the prevention of the formation of free radicals or the conversion of oxidants to less toxic species (Kalantar-Zadeh, Lee & Block 2004). Well known vitamin antioxidants are vitamins A, C and E. β -carotene is a pro-vitamin A molecule that can be cleaved by β -carotene di-oxygenase enzyme into two molecules of vitamin A, hence offering antioxidant activity (Bjelakovic et al. 2007). Other minerals including selenium, manganese and zinc are cofactors for antioxidant enzymes (Hennig et al. 1999; Kalantar-Zadeh, Lee & Block 2004). Fruits, vegetables and also whole grain products are rich in antioxidant vitamins and minerals (Tabak et al. 2005).

Antioxidants provide a defensive system in which the survival and development of cells in an oxidant-containing environment is possible. Currently, information on the

relationship between asthma and antioxidants in children is scarce although some studies in adults have found modest protective effects (Troisi et al. 1995).

1.7.1.1 The role of antioxidants in modification of immune responses

Antioxidants are important in protecting against oxidative stress (Sackesen et al. 2008). Nuclear transcription factor kappa B (NF κ B) acts as the main controller of inflammation which is activated by reactive oxygen species (Sackesen et al. 2008). Antioxidants in the diet can help to scavenge reactive oxygen species by inhibiting NF κ B-mediated innate immune response. In vitro studies have shown that vitamin C through suppression of NF- κ B activity has a direct effect on innate immune activation. High circulating levels of serum vitamin C are associated with enhanced neutrophil function. Vitamin C can reduce the levels of TNF α , IL-6 and IL-8 in acute pancreatitis. Vitamin E has been shown to inhibit: reactive oxygen species production by leukocytes and cyclo-oxygenase activity. β -carotene has strong antioxidant action through quenching of reactive oxygen species (Kirkham & Rahman 2006; Sackesen et al. 2008). β -carotene can also inhibit NF- κ B activity in lipopolysaccharides exposed cells (cells infected with gram negative bacteria) (Sackesen et al. 2008).

1.7.1.2 Mechanism of action of antioxidants on asthma

Because asthma is characterized by the chronic inflammation of the airways, it has been postulated that epithelial injury due to oxidant–antioxidant imbalance may represent a major pathogenic mechanism in asthma (Tan-Un, Chang & Chan-Yeung 2004).

Reactive oxygen species are continuously produced in the human body (Kalantar-Zadeh, Lee & Block 2004). The survival and development of cells in an oxidant-containing environment require an antioxidant defence system. Inflammatory cells generate considerable amounts of oxidants on the epithelial surfaces, leading to epithelial cellular injury. Sources of reactive oxygen species include primarily the membrane associated NADPH oxidase dependent complex, the cytosolic xanthine oxidase system, and the mitochondrial respiration chain (Yeum et al. 2009). Superoxide anion is then converted to hydrogen peroxide (H_2O_2), either spontaneously or under the influence of superoxide dismutases (SOD) (Emecen et al. 2009; Nauta et al. 2008). Both O_2 and H_2O_2 are moderate oxidants and are critical for the formation of potent cytotoxic radicals in biological systems through their interaction with other molecules. For instance, the lysosomal enzymes myeloperoxidase (MPO) from neutrophils and monocytes/macrophages and the eosinophil peroxidase (EPO) catalyse the oxidation of halides (Cl_2 , Br_2 , and I_2) by H_2O_2 to form hypohalous acids ($HOCl$ or $HOBr$) (Southam et al. 2007). MPO produces predominantly hypochlorous acid ($HOCl$) whereas EPO produces more hypobromous acid ($HOBr$). Hypohalous acid production is important in the host defense against infectious agents, but during this reaction the hydroxyl radical ($OH\cdot$) is also produced, which is a powerful and indiscriminate oxidant. Eosinophils possess several times greater capacity for generating oxidants than neutrophils, and the eosinophil peroxidase content of eosinophils is several times higher than that of MPO in neutrophils (Emecen et al. 2009; Nauta et al. 2008). Myeloperoxidase and eosinophil peroxidase derived reactive oxygen species can also interact with nitrite (NO_2^-) and H_2O_2 leading to the formation of reactive nitrogen species (RNS; nitrosants) (Southam et al. 2007). A powerful oxidant, the radical peroxynitrite

(ONOO₂), is produced from the reaction between O₂ and nitric oxide (NO) which is increased in the asthmatic airways (Yeum et al. 2009).

The inflammatory cells recruited to the asthmatic airways have special capacity for producing oxidants. Once recruited in the airspaces, inflammatory cells may become activated and generate reactive oxidants in response to various stimuli (Emecen et al. 2009; Nauta et al. 2008). Activated eosinophils, neutrophils, monocytes, and macrophages, and also resident cells such as bronchial epithelial cells, can generate oxidants. Antioxidant–oxidant imbalances in bronchoalveolar fluid may contribute to oxidative stress in respiratory disease, since an excessive free radical generation in the blood of children suffering from asthma has been shown (Shanmugasundaram, Kumar & Rajajee 2001b) (Figure 4).

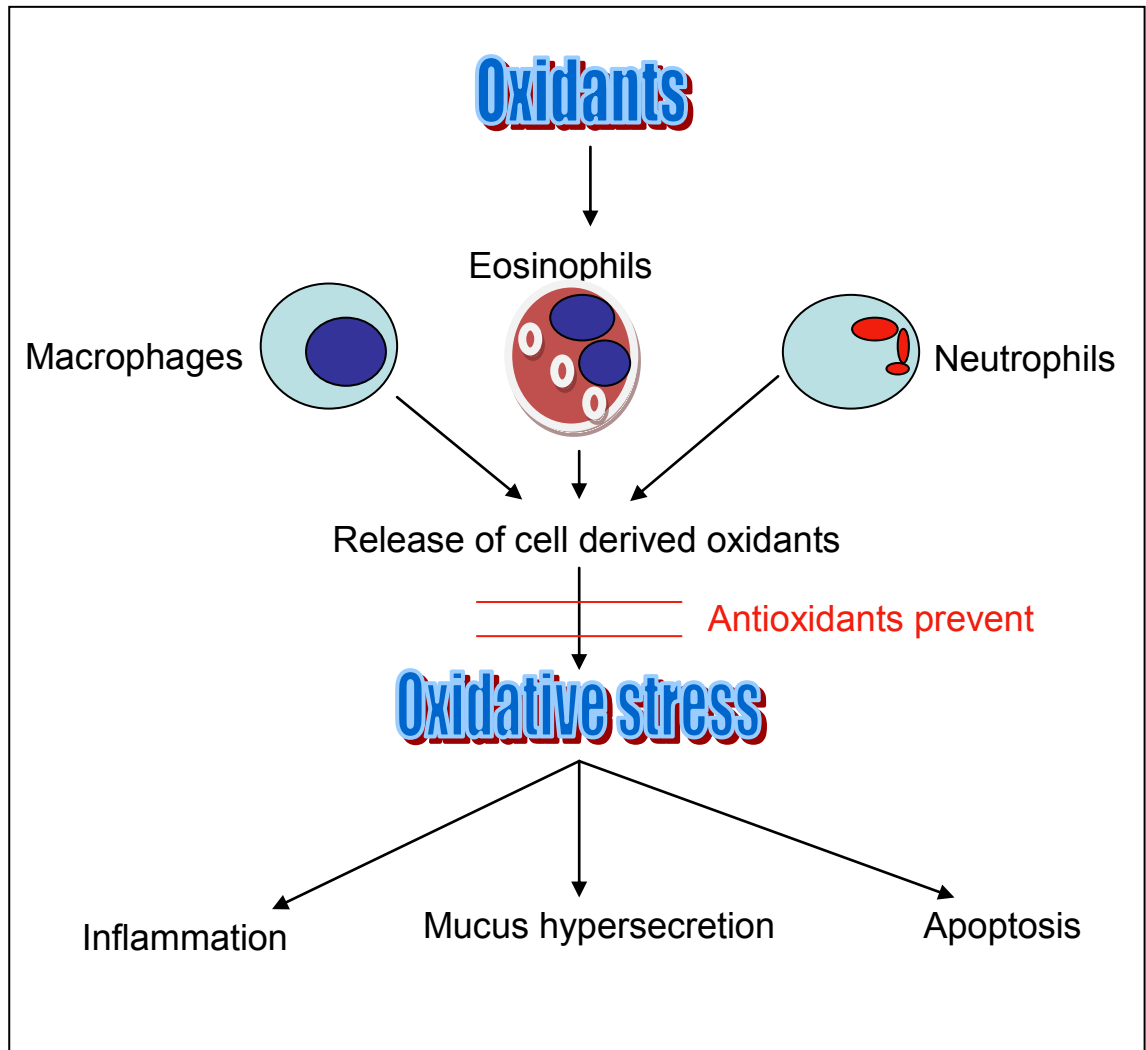


Figure 4- Antioxidant–oxidant imbalances in bronchoalveolar fluid may contribute to oxidative stress in respiratory system

(Compiled by the candidate)

It is suggested that vitamin E and β -carotene in the diet may reverse the imbalance of oxidants/antioxidants and thereby reduce airway inflammation and the severity of asthma in some patients (Butland, Strachan & Anderson 1999). As a part of my project, I investigated the association between asthmatic symptoms and consumption of vitamins A, C, and E, β -carotene and zinc in adolescents at 14 years of age.

1.7.1.3 Epidemiological studies on association of asthma and antioxidants

In a study conducted on young adult smokers Omenaas and colleagues (2003) found that dietary vitamin C intake was inversely related to symptoms of cough and wheeze, indicating that there may be a protective antioxidant effect. The same outcome was observed in another study in which it was demonstrated that high dietary intake of vitamin C and the subsequent rise in serum concentrations of this vitamin had a protective effect against respiratory symptoms in asthmatic children (Forastiere et al. 2000). However, in a large cohort study of adult women, no such protective effect of vitamin C was observed on the symptoms of asthma (Dow et al. 1996). Differences in smoking prevalence, gender and/or age of the subjects may explain the observed differences between these studies.

1.7.1.4 Vitamins A, C, and E

Regardless of the nature of the study whether being population based or in-vitro, there is either limited evidence or mixed results on the relationship between asthma and different antioxidants (McKeever & Britton 2004). For instance in one study it was found that asthmatic adult patients have a lower intake of vitamins A, C, and E compared with non-asthmatic subjects, although after adjusting for smoking, age, and sex with the dependent variable of forced expiratory volume in 1 second (FEV1) only vitamin C remained in the model (de Luis et al. 2005).

The free alcohol form of vitamin A (retinol) can be reversibly converted by enzymatic activity to the active form of vitamin A, retinal, in a variety of tissues. Retinal can be further converted to the potent transcription factor, retinoic acid, but this reaction is not reversible. Except in the retina, retinal concentrations are normally very low in all tissues due to enzyme kinetics that favour either its re-conversion to retinol, or transformation to retinoic acid. In addition to its enzymatic conversion from retinal, retinoic acid may be extracted by most cells from the plasma where it is transported bound to albumin. Once in the cell, retinoic acid is bound to

cellular retinoic acid binding protein (De Oliveira et al. 2009; Emecen et al. 2009). The latter may facilitate the delivery of retinoic acid to responsive transcription factor binding sites, where it binds with vitamin D3 and thyroid hormones. Vitamin A can act as a chain breaking antioxidant by combining with peroxy radicals, before these radicals can propagate peroxidation in the lipid phase of the cell and generate hydroperoxides. Retinol was shown to be an effective peroxy radical scavenger by its inhibition of peroxidation in a homogeneous solution of methyl linoleate and in model phosphatidylcholine liposomes (De Oliveira et al. 2009).

Vitamin C is an effective antioxidant of the hydrophilic phase. It can also protect from lipid peroxidation in the hydrophobic compartments by scavenging lipid peroxides or reducing tocopherol radicals to tocopherols. At low concentrations and in the presence of transition metal ions such as iron (Fe), vitamin C facilitates NADH dependent lipid peroxidation through reduction of Fe (III) to Fe (II) (Yeum et al. 2009).

Vitamin E is a lipophilic chain-breaking antioxidant that acts by stopping the chain reaction involved in lipid peroxidation (Greene 1999). Vitamin E is the major antioxidant soluble in lipids protecting cellular membranes and lipoproteins against peroxidation. The reaction of α -tocopherol with free radicals generates tocopherol radicals, which can be reduced by vitamin C or reduced glutathione (GSH).

Information on the relationship between asthma and antioxidants other than vitamins C and E and some cations (zinc and manganese) in children is scarce. In an investigation in adult asthmatics, asthma was associated with a low dietary intake of fruit, antioxidants, vitamin C and manganese (Patel et al. 2006). People who consumed more than 46.3 g/day of citrus fruits had a reduced risk of diagnosed asthma OR 0.59 (95% CI 0.43 to 0.82) and symptomatic 0.51 (95% CI 0.33 to 0.79) asthma compared to those who had zero intake (Patel et al. 2006). Dietary vitamin C and manganese were inversely and independently associated with symptomatic asthma (adjusted OR per quintile increase 0.88 (95% CI 0.77 to 1.00) for vitamin C and 0.85 (95% CI 0.74 to 0.98) for manganese). Adjusted plasma levels of vitamin C were significantly lower in symptomatic cases than in controls (54.3 v 58.2 μ mol/l, $p = 0.003$) (Patel et al. 2006). Some papers in adults have found modest beneficial

effects of vitamins C and E, some cations (zinc and manganese) and other antioxidants (Castro-Rodriguez et al. 2008; Willers et al. 2007).

1.7.1.5 Selenium

Selenium is also involved in antioxidant defenses as a coenzyme in glutathione peroxidase (GSH), the enzyme believed to be an important component of the pulmonary antioxidant system (Chatzi et al. 2007; Stone, Hinks & Beasley 1989). Most of the selenium in mammalian tissues is associated with the amino acids selenocysteine and selenomethionine in proteins (Bjelakovic et al. 2007). Glutathione peroxidase does not only protect cells against damages by free radicals, but also protects membrane lipids against such oxidation generated by peroxides and permits regeneration of membrane lipid molecules through re-acylation (Chatzi et al. 2007).

Further evidence of the role of nutrients on asthma was provided by Stone and colleagues who found that patients with symptomatic asthma had a reduced plasma selenium concentration (Stone, Hinks & Beasley 1989). The asthmatic patients had significantly lower concentrations of selenium measured in plasma ($p < 0.001$) and whole blood ($p < 0.001$), and the OR were highly significant with a 3.54 fold increased probability of asthma for the lower range of plasma and 5.08 fold for whole blood selenium concentrations (Stone, Hinks & Beasley 1989). However, in a case-control study conducted by Shaheen et al., the protective role of antioxidants on asthmatic symptoms in adults was shown to be weak, as the intake of selenium was also negatively associated with asthma (OR per quintile increase 0.84 [0.75 to 0.94]; $p = 0.002$) (Shaheen et al. 2001). This could be due to several factors including reluctance of participants to complete a long food frequency questionnaire and the consequent low response rate as stated by these investigators.

Baker and Ayres suggest that sub-optimal nutrient intake of antioxidant vitamins A, C and E, and selenium in adults may enhance asthmatic inflammation, consequently contributing to bronchial hyperreactivity (Baker & Ayres 2000).

1.7.1.6 Zinc

Zinc is an essential dietary factor with antioxidant properties present in all organs, tissues, and body fluids, and mediates a wide variety of physiological processes (Zalewski et al. 2005). With an average presence of 3 grams in the human body it is the second most abundant trace element after iron which has an estimated around 4 grams (Amerio et al. 2003). According to the American Dietetic Association, the recommended daily allowances of zinc are 5 mg/day for infants, 10 mg/day for children, 15 mg/day for teenagers, adults, and pregnant women, and 16–19 mg/day for lactating women (Arsenault & Brown 2003). Zinc is normally obtained from red meat and other animal proteins. Other sources of zinc are sea food, dairy food, cereals and nuts (Arsenault & Brown 2003). Most vegetables are not ready sources of zinc due to the presence of phytate, the principal storage form of phosphorus in many plant tissues, as phytate inhibits zinc absorption (Arsenault & Brown 2003).

Diets low in animal protein and rich in phytate contribute to the high incidence of zinc deficiency in many developing countries. Another cause of human primary zinc deficiency can be low zinc content of soils (Lambein et al. 1994). The bulk of body zinc is tightly bound within cellular enzymes and zinc finger proteins, the protein domains that can bind to deoxyribonucleic acid (DNA) (Zalewski et al. 2005). This fixed pool of zinc turns over very slowly and is mainly responsible for housekeeping functions in cellular metabolism and gene expression. The remaining 10–15% of zinc (labile zinc) comprises more dynamic pools that are readily depleted in zinc deficiency (Arsenault & Brown 2003).

Henning et al (1999) describe the role of zinc as an antioxidant. They propose that zinc inhibits oxidative stress-responsive events in endothelial cells after treatment of these cells by either fatty acids or Tumour Necrosis Factor (TNF)- α (Hennig et al. 1999). The antioxidant property of zinc has also been described by Powell (2000) who demonstrated that zinc is capable of reducing post ischemic injury in a variety of tissues through a mechanism that might involve the antagonism of copper reactivity. As mentioned earlier (section 1.7.1.1) antioxidant–oxidant imbalances in bronchoalveolar fluid may contribute to oxidative stress in respiratory disease

(Shanmugasundaram, Kumar & Rajajee 2001b). Zinc therefore, as an antioxidant, may have a protective effect in contributing to the establishment of antioxidant–oxidant balance in bronchoalveolar fluid.

The epithelial airway is vulnerable to a range of oxidants derived internally. Reactive oxygen species are continuously produced in our bodies as a result of biochemical reactions, or externally from atmospheric oxidants and from inflammatory cells during acute or chronic inflammation of the airways (Shanmugasundaram, Kumar & Rajajee 2001a).

To combat these oxidants, epithelial airway and its secretions contain a variety of anti-oxidants including catalase, vitamins C and E, glutathione, and two forms of superoxide dismutase (Arsenault & Brown 2003). Zinc is a component of a major anti-oxidant enzyme in epithelial airway (Ho et al. 2004; Zalewski et al. 2005). Zinc is reported to be essential for many components of the immune system including phagocytic function, cellular immunity, humoral immunity, and NK cell function (Ho et al. 2004). Some of the anti-oxidant effects of Zn are due to stabilization of sulphhydryls and membrane lipids and suppression of nitric oxide production (Zalewski et al. 2005). Zinc is also a component of a major anti-oxidant enzyme in airway epithelium, Cu/Zn superoxide dismutases (SOD). Although the role of Zn in Cu/Zn SOD is unclear, its removal is known to promote catalysis of peroxynitrite-mediated tyrosine nitration, resulting in protein oxidation and consequent cellular damage (Zalewski et al. 2005).

Zinc is essential for many components of the immune system including phagocytic function, cellular immunity, humoral immunity, and natural killer cell function (Ho et al. 2004). Mast cells have a well-developed mechanism for uptake of zinc as well as mechanisms to concentrate zinc into their granules (Ho et al. 2004; Zalewski et al. 2005). The role of granular zinc in mast cells is unclear but it may act in a feedback loop to down-regulate secretion of histamine and other mediators. Zinc exerts a potent anti-apoptotic effect for immature T cells in the mouse thymus; zinc regulates the balances between CD^{4+} and CD^{8+} T cells and between Th_1 and Th_2 subsets. Zinc is required for secretion of various T-dependent interleukins and cytokines including IL-1, IL-2, IL-4, and IFN- γ (Zalewski et al. 2005).

Due to the important role of zinc as an antioxidant, we investigated a possible association between dietary zinc intake and asthma in adolescents.

1.7.1.7 β -carotene

β -Carotene belongs to a family of molecules called Carotenoids. These molecules have strong antioxidant actions therefore can be useful for restricting the damaging excess of free radicals in the body (Bjelakovic et al. 2007). The most common carotenoids in biological systems are lycopene, α - and β -carotene, lutein and β -cryptoxanthin (Wood and Gibson 2009). β -carotene is composed of two retinyl groups, and is broken down to retinal, a form of vitamin A in the mucosa of the small intestine by β -carotene dioxygenase.

β -carotene can be stored in the liver and converted to vitamin A as needed, thus making it a provitamin (Bjelakovic et al. 2007). β -carotene is lipid soluble and is concentrated in cell membranes; it protects against lipid peroxidation of membranes and has demonstrated antioxidant effects (Greene 1999). Studies show β -carotene scavenge free radicals to prevent lipid peroxidation in chest disease (Genovese et al. 2005; Klings & Farber 2001), however, there is no evidence of the possible role of β -carotene in asthma in children.

1.7.1.8 Flavonoids

Flavonoids are a class of secondary plant phenolics with significant antioxidant properties. They are most concentrated in fruits, vegetables, wines, teas and cocoa (Heim, Tagliaferro et al. 2002). A high flavonoid intake has been associated with lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men and reduced risk of coronary heart disease by 38% in postmenopausal women (Heim, Tagliaferro et al. 2002). The protective effects of flavonoids in biological systems are attributed to their capacity to transfer electron

free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidase (Heim, Tagliaferro et al. 2002).

1.7.2 Fatty acids

Fatty acids are the building blocks of triglycerides and phospholipids (predominantly in cell membrane). Fatty acids occur in free form or are esterified to other lipids.

Over 70 different fatty acids have been isolated from cells, and they all possess a long hydrocarbon chain with a terminal carbonyl group located at one end (Schubert et al. 2007). The long chain may be either saturated or unsaturated, depending on the presence or absence of double bonds.

The adipocytes are a class of specialized cell types for the storage and release of fatty acids. Adipocytes are unique in that they can accommodate without deleterious effects the massive storage of triacylglycerol during energy profusion and releases free fatty acids into the plasma for the use by other tissues during times of energy need (Ogwok, Muyonga & Sserunjogi 2008). This process of fatty acid uptake and storage balanced by lipolysis is a highly regulated process that takes cues from nutritional and efferent signals to store and supply energy as the body dictates. The adipocyte has a unique cellular organization as well, with greater than 90% of the cell volume being triacylglycerol. These results in limited cytosolic space and an adjacent endoplasmic reticulum, nuclear, plasma membrane interface (Howe et al. 2006; Radmark et al. 2007).

This geometry may accommodate the transport of hydrophobic molecules, such as fatty acids and fatty acetyl coenzyme-A (a key intermediate molecule in aerobic metabolism of lipids and carbohydrates) to and from the membrane during uptake and lipolysis. The insolubility of fatty acids may also be accommodated by intracellular carriers such as the fatty acid binding proteins and acetyl coenzyme-A binding proteins (Bolte et al. 2006).

Differences between the 70 fatty acids are primarily in the length of the hydrocarbon chain, and the number and position of double bonds. Nearly all the fatty acids in biological systems have an even number of carbon atoms, typically 14 to 24 carbons with 16 and 18 carbon atoms being the most common found in the body (Murakami et al. 2007).

The primary sources of fatty acids are dietary (mainly fish, flaxseeds, walnut, and canola) and mobilization from cellular stores. Fatty acids from the diet can be delivered from the gut into the cells via transport in the blood stream. Fatty acids are stored in the form of triacylglycerols primarily within adipocytes of adipose tissue. In response to energy demands, the fatty acids of stored triacylglycerols can be mobilized for use by peripheral tissues. Nutritionally, a majority of saturated fatty acids tend to lead to higher levels of blood cholesterol, because saturated fatty acids have a regulating effect on cholesterol synthesis and unsaturated fatty acids do not (Socha et al. 2007).

The increase in childhood allergy and asthma rates may be partially attributable to temporal trends in dietary intake, particularly during pregnancy and early childhood (Gold et al. 2006). These trends include decreases in intake of foods containing n-3 polyunsaturated fatty acids (PUFAs), such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in fish, nuts, or seeds and increases in intake of foods containing n-6 fatty acids, such as linoleic acid (LA) and arachidonic acid (AA), found in vegetable oils and animal products (Gold et al. 2006). Therefore, there is increasing evidence that a diet, with high levels of n-3 fatty acids has a protective effect against cough and wheeze both in children and adults (Castro-Rodriguez et al. 2008; Peat et al. 2004). Consumption of oily fish (which has a high content of very long chain n-3 fatty acids such as EPA and DHA) in the diet is also shown to be associated with a reduced prevalence of asthma (Hodge et al. 1996; Hodge et al. 1998). N-3 may reduce asthma prevalence by influencing the development of allergic sensitization through reducing the formation of immunoglobulin E (IgE) in lymphocytes (Baker & Ayres 2000; LeGros et al. 1990; Mosmann & Coffman 1989).

An epidemiological study in children has shown inverse associations of asthma with intake of whole grain products and fish (Tabak et al. 2005). For current asthma the adjusted odds ratios for the independent associations with whole grains was shown as 0.46 (95% CI 0.19 to 1.10), and for fish 0.34 (95% CI 0.13 to 0.85) (Tabak et al. 2005). Also for atopic asthma with BHR the adjusted odds ratios for whole grains was shown as 0.28 (95% CI 0.08 to 0.99) and for fish 0.12 (95% CI 0.02 to 0.66) (Tabak et al. 2005). Therefore, whole grain products and fish played a protective role against current asthma and atopic asthma in this study.

Early-life exposure to n-3 fatty acids has been hypothesized to decrease asthma or allergy risk through immune modulation, decreasing T-Helper type2 cytokine (eg, IL-13 and IL-4) secretion, subsequent IgE production, and the risk of chronic allergic inflammation, including airway inflammation (Gold et al. 2006). In one study, all neonates whose mother had been taking fish oil supplementation (n = 40) during their pregnancy, had significantly higher proportion of total n-3 PUFAs in their erythrocyte membranes (mean=17.75 ± 1.85 SD), compared with the control group (n=43) (mean =13.69 ± 1.22 SD) ($p < 0.001$). Conversely, total neonatal n-6 PUFA composition was significantly lower: mean =25.21% ± 1.82 SD in the fish oil compared with the control group: mean=29.50 ± 1.35 SD ($p < .001$). Also the group with higher n-3 PUFA had lower cytokine responses (IL-5, IL-13, IL-10, and IFN- γ) to different allergens compared with the control group (Dunstan et al. 2003).

In another study, children who received n-3 fatty acids supplementation from six months of age had fewer wheezes in the first 18 months of life than control subjects (Mihirshahi et al. 2003). If the child was breast-feeding before the age of 6 months, no supplement was given, because the concentration of n-3 in breast milk was believed to be equivalent to that of the supplement. However, if bottle-feeding was introduced before the age of 6 months, the parents were asked to add the n-3 supplement to the child's formula (Mihirshahi et al. 2003). This could be due to the effects of dietary and free fatty acids in milk on the immune system (Mihirshahi et al. 2003). However, later on when these children were five years of age, it was shown that fatty acid exposure was not associated with any respiratory or allergic outcomes (Almqvist et al. 2007).

Several studies have shown that n-3 polyunsaturated fatty acids might possess anti-inflammatory effects in patients with inflammatory bowel disease and in animal models of colitis (Babcock et al. 2002; Hegazi et al. 2006; Stark, Lim & Salem 2007). These anti-inflammatory properties may be mediated by a decrease in inflammatory eicosanoids and decreased secretion of inflammatory cytokines (Caughey et al. 1996). On the contrary, other studies have shown that fish oil induces the pro-inflammatory cytokine tumour necrosis factor (TNF) and inhibits the secretion of the anti-inflammatory cytokine, interleukin-10 (IL-10) (Babcock et al. 2002). The Cochrane review of n-3 and n-6 fatty acids supplementation on atopic dermatitis and asthma is inconclusive, with only one trial reporting a statistically significant effect of n-6 supplementation on lowering serum immunoglobulin E (IgE) levels ($p < 0.01$) in atopic participants (Anandan et al. 2009). N-3 supplementation effects in immunological responses are reported as either short term or inconsistent (Anandan et al. 2009).

Therefore, the role of fatty acids in the modulation of chronic diseases is controversial. In this research the role of fatty acids (both from dietary and red blood cells) on the modulation of asthma will be examined.

1.7.2.1 N-3 and n-6 fatty acids structure

The n-3 fatty acids (n-3) are long-chain polyunsaturated fatty acids ranging from 18 to 22 carbon atoms in chain length with the first of many double bonds beginning at the third carbon from the methyl end of the fatty acid structure (Holub 2002) (Figure 5).

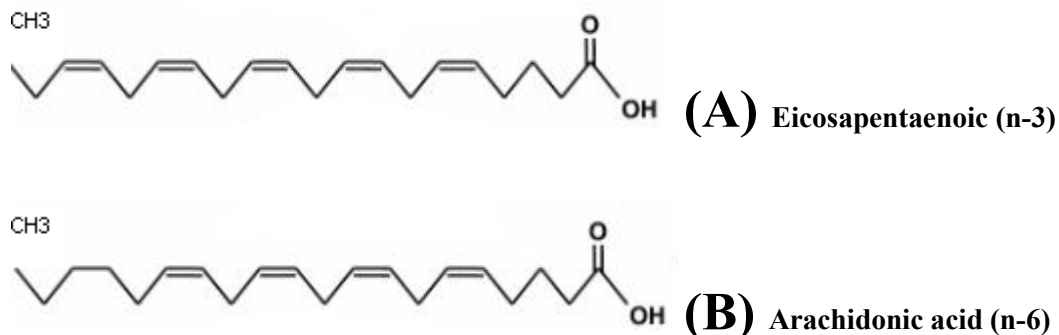


Figure 5- Demonstration of differences between chemical structures of n-3 and n-6 fatty acids.

A) Eicosapentaenoic acid (EPA) C20:5 (n-3),

B) Arachidonic acid (AA) C20:4 (n-6).

Fish and fish oil-based n-3 polyunsaturated fatty acids, consist of docosahexaenoic acid or DHA and eicosapentaenoic acid or EPA. DHA and EPA can be abbreviated as 22:6n-3 (i.e., 22 carbon atoms with 6 double bonds) and 20:5n-3, respectively. The number before the colon indicates the number of carbon atoms in the fatty acid chain and the number after the colon indicates the number of double bonds between adjacent carbon atoms (unsaturated sites) in the structure.

DHA contains 22 carbon atoms and 6 double bonds and fish is one of the richest sources of this nutrient (Willers et al. 2007). EPA contains 20 carbon atoms and 5 double bonds (Holub 2002; Marangoni et al. 2007). Walnuts are rich sources of EPA, in a recent study levels of EPA were significantly raised in blood lipids by the intake of four walnuts a day in humans (Marangoni et al. 2007).

Linoleic acid (LA) 18:2n6 and α -linolenic acid (ALA) 18:3n3 are essential fatty acids. Eicosapentaenoic acid (EPA) 20:5n3 and docosahexaenoic acid (DHA) 22:6n3 are not essential nutrients because EPA can be synthesised from ALA and DHA from EPA (DHA can be reconverted into EPA) (Holub 2002; Marangoni et al. 2007).

1.7.2.2 Sources of n-6 and n-3

Foods that are rich in n-6 fatty acids include poppy seed oil, palm oil, sunflower oil, soybean oil, coconut oil, egg yolks, peanut oil, rice bran oil, wheat germ oil, grape seed oil, macadamia oil, pistachio oil and sesame oil (Cunnane, Trotti & Ryan 2000).

The increased consumption of polyunsaturated fats in Australia in recent years followed by the campaigns to reduce heart disease (by reducing saturated fat consumptions) could be partly responsible for the increase in the prevalence of asthma (Haby et al. 2001). Polyunsaturated fats are a rich source of n-6 fatty acids, such as linoleic acid and they can increase the synthesis of prostaglandin E2 (Marangoni et al. 2007). The net effect is the increase in the risk of inflammation, which may increase the risk of asthma (Black & Sharpe 1997; Bolte et al. 2006; Haby et al. 2001).

Foods that are rich in n-3 fatty acids include whole grains, fresh fruits and vegetables, fish, olive oil, garlic (Marangoni et al. 2007). Beef and lamb has a high concentration of docosapentaenoic acid (DPA) (Mann et al. 2003). N-3 fatty acids may have the opposite effect of n-6 by inhibiting the formation of prostaglandin E2 and protecting against inflammation (Haby et al. 2001; Marangoni et al. 2007), however, this is controversial.

The incorporation of linoleic acid (18:2n6) and α -linolenic acid (18:3n3) from the diet into serum and tissue phospholipid stores appears dependent on the ratio of n6: n-3 in the diet, rather than the absolute amount of either fatty acid present (Meyer et al. 2000).

N-3 fatty acids may have the opposite effect of n-6 by inhibiting the formation of prostaglandin E2 and protecting against inflammation (Haby et al. 2001; Marangoni et al. 2007), however, this is controversial.

The n-6 fatty acids, dihomo gamma-linolenic acid (DGLA) 20:3n-6 can be converted to either the anti-inflammatory prostaglandin E1 or into arachidonic **acid**

(AA), a precursor of prostaglandin E2. Conversion of DGLA into prostaglandin E1 does not require any enzymes, but conversion of DGLA into AA requires the enzyme delta-5 desaturase. In diets high in n-3, most of the delta-5 desaturase will be used in the n-3 pathway; few delta-5 desaturase will be available to convert DGLA into arachidonic acid, and subsequently, prostaglandin E2. DGLA ends up being converted into the anti-inflammatory prostaglandin E1 and inflammation is therefore decreased (Babcock et al. 2004; Calder 2006; Calderon & Kim 2007; Lands 2008).

In a diet low in n-3 fatty acids, large quantities of delta-5 desaturase enzymes are available to convert DGLA into AA. The available AA is then converted into the inflammatory prostaglandin E2. Thus, the more n-3 fatty acids present in our body, the fewer enzymes are available for converting n-6 fatty acids into the inflammatory prostaglandins. A balance of n-6 and n-3 fatty acids is therefore essential for proper health. However, the typical Western diet has evolved to be high in n-6 and low in n-3 fatty acids (Calder 2006; Calderon & Kim 2007; Lands 2008).

1.7.2.3 Fatty acids and the immune system

The effects of the fatty acids on the immune system and its responses are complex, because they depend on many different factors including the type and concentration of fatty acids, cell types and species of experimental animals (Almqvist et al. 2007; De Pablo & De Cienfuegos 2000; McKeever 2007; Wong 2005). Therefore, it is not surprising that several conflicting results on the effects of dietary and free fatty acids on the immune system have been reported (Almqvist et al. 2007; De Pablo & De Cienfuegos 2000; McKeever 2007; Wong 2005). Linoleic acid (n-6 fatty acid) is a precursor of arachidonic acid, which can be converted to prostaglandin E2 (PGE2), whereas eicosapentaenoic acid (n-3 fatty acid) inhibits the formation of PGE2. Prostaglandin E2 (PGE2) acts on T-lymphocytes to reduce the formation of interferon- γ (IFN- γ) without affecting the formation of interleukin-4 (IL-4). This may lead to the development of allergic sensitization, since IL-4 promotes the synthesis of immunoglobulin E (IgE), whereas IFN- γ inhibits the synthesis of immunoglobulin E (IgE) (Bolte et al. 2006; Haby et al. 2001; LeGros et al. 1990; Mosmann & Coffman 1989; Nagel & Linseisen 2005; Oddy et al. 2004a). Therefore, an increase in the ratio

of n-6:n-3 fatty acids associated with a change in diet may result in a higher synthesis of IgE by lymphocytes and this in turn can explain the increase in the prevalence of allergic reactions including asthma in recent decades in Western countries due to changes in diet habits.

Results from observational studies support the hypothesis that a diet that includes fish with high levels of n-3 fatty acids has a protective effect against cough and wheeze both in children and adults (Bolte et al. 2006; Broadfield et al. 2004; Mickleborough, Ionescu & Rundell 2004; Peat et al. 2004; Wong 2005). Inclusion of oily fish in the diet is also proposed to be associated with a reduced prevalence of asthma (Oddy et al. 2004b; Wong 2005). On the other hand, it is proposed that a high margarine intake (which is high in n-6) significantly increases the risk of onset of asthma in adulthood (Bolte et al. 2006; Haby et al. 2001; Nagel & Linseisen 2005).

Many mechanisms involved in modulation of the immune system by fatty acids are either unknown or poorly understood. Four factors that are most frequently cited in the literature are: (i) membrane fluidity, which is modified as a consequence of changes in phospholipid composition; (ii) eicosanoid production from long-chain fatty acids, showing different biological properties depending on their precursors; (iii) formation of lipid peroxides, which are toxic to the cells; and (iv) influence on the regulation of the expression of genes encoding proteins that participate in cellular responses (De Pablo & De Cienfuegos 2000; Hasala et al. 2008; Park et al. 2007).

Some studies suggest that n-6 fatty acids have a detrimental effect on allergic responses and asthma (McKeever 2007), however, a clinical trial of n-6 fatty acids supplementation in adults found that some n-6 fatty acids, such as γ -linolenic acid (GLA), might have similar effects to those of n-3 fatty acids on the decrease in proliferation of activated lymphocyte (Thies et al. 2001). Also increased foetal levels of arachidonic acid (AA) has similar effects as increased foetal levels of EPA in attenuating of immunologic responses, specifically a reduction in allergen-stimulated lymphocyte proliferation and IFN- γ production (Gold et al. 2006). These researchers proposed a protective role for n-6 fatty acids against allergic reactions and asthma while the others (McKeever 2007), suggest a detrimental role for these fatty acids on asthma.

In one study (Salam et al. 2005) monthly maternal consumption of oily fish was significantly protective against persistent asthma in five year old children born to mothers with a history of asthma (OR = 0.45; 95%CI = 0.23–0.91). No associations were found in the children born to non-asthmatic mothers. In another study maternal fish consumption was shown to be beneficially associated with doctor-confirmed eczema in 5-year-old children (OR = 0.57; 95%CI = 0.35–0.92) (Willers et al. 2007).

Linoleic acid is a precursor of arachidonic acid, which can be converted to prostaglandin E₂ (PGE₂), whereas α -linolenic, a precursor of eicosapentaenoic acid, inhibits the formation of PGE₂. Prostaglandin E₂ (PGE₂) acts on T-lymphocytes to reduce the formation of interferon- γ (IFN- γ) without affecting the formation of interleukin-4 (IL-4). This may lead to the development of allergic sensitization, since IL-4 promotes the synthesis of immunoglobulin E (IgE), whereas IFN- γ inhibits the synthesis of immunoglobulin E (IgE) (Bolte et al. 2006; Haby MM et al. 2001; LeGros et al. 1990; Mosmann & Coffman 1989; Nagel & Linseisen 2005; Oddy et al. 2004a) (Figure 6). Arachidonic acid is abundant in phospholipids from meat and that acts as a substrate for 5-Lipoxygenase (5-LO). 5-LO catalyses the first two steps in the biosynthesis of leukotrienes, a group of pro-inflammatory lipid mediators derived from arachidonic acid. Leukotriene antagonists are used in the treatment of asthma (McNamara, Sullivan & Richtand 2008; Radmark et al. 2007).

Polyunsaturated fatty acids are major constituents of the membrane phospholipids of inflammatory cells (Riediger et al. 2009). During inflammation, intracellular concentration of calcium ions (Ca²⁺) increases and the ionized calcium binds to calmodulin (a calcium-dependent regulatory protein) (Wong 2005). When the four binding sites of calmodulin are fully occupied by Ca²⁺, calmodulin undergoes a conformational change that enables it to phosphorylate an active site residue (Ser⁵⁰⁵) within the catalytic site of phospholipase A₂ (Hegazi et al. 2006; Wong 2005). The activated phospholipase A₂ hydrolyzes the second-position ester bond of the phospholipids and releases n-6 fatty acid (linoleic acid 18:2n6), which is then converted into pro-inflammatory arachidonic acid (AA) based eicosanoid (Hegazi et al. 2006) (Figure 7).

Depending on the levels of dietary intake, n-3 fatty acids compete with and displace n-6 fatty acids for the acylation sites in the cellular membranes (Mickleborough et al. 2009). It has been shown that the saturation of n-3 in cell membranes can significantly increase within two weeks supplementation and the levels of eicosapentaenoic acid could reach peak accumulation after six weeks of supplementation (Mickleborough et al. 2009; Wong 2005). Dietary fat intake may change the membrane composition of fatty acids and modulate the types of eicosanoids produced in the pathway, thus influencing the inflammatory response of the cells. N-6 is metabolized into arachidonic acid through a process of desaturation and elongation (Wong 2005) (Figure 7).

The delta-5 desaturase is an enzyme that catalyzes the last step of the conversion of dihomo- γ -linolenate. The presence of eicosapentaenoic acid acts as a negative allosteric effector of delta-5 desaturase, which is the rate-limiting enzyme of arachidonic acid metabolism. Hence, eicosapentaenoic acid limits the direct precursors of the leukotrienes (Schmitz & Ecker 2008).

In this research along with the individual fatty acids (α -linolenic acid, parinaric acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, linoleic acid, arachidonic acid and docosatetraenoic acid), total n-3 and total n-6, the effects of the ratio of n-6:n-3 on asthma and asthmatic symptoms were investigated.

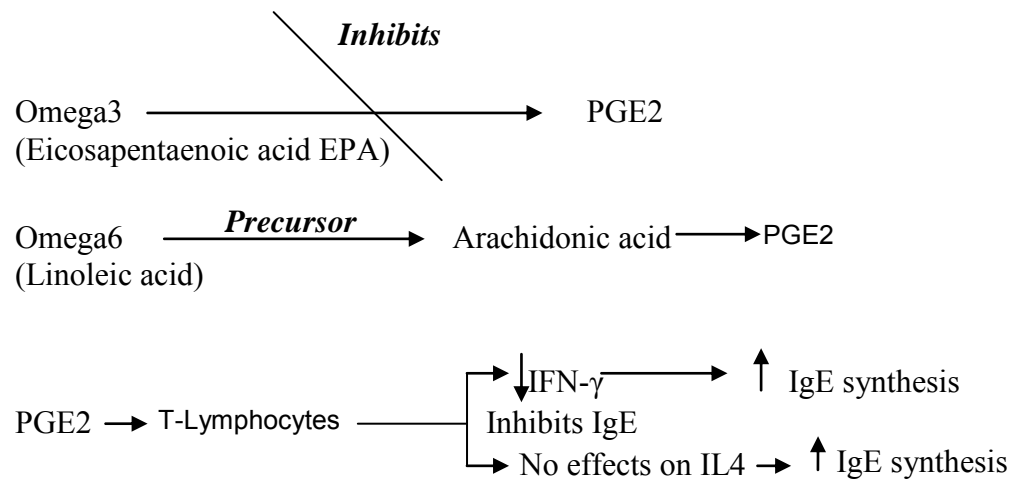


Figure 6- Proposed effects of n-3 and n-6 fatty acids on IgE synthesis.
 (Compiled by the candidate)

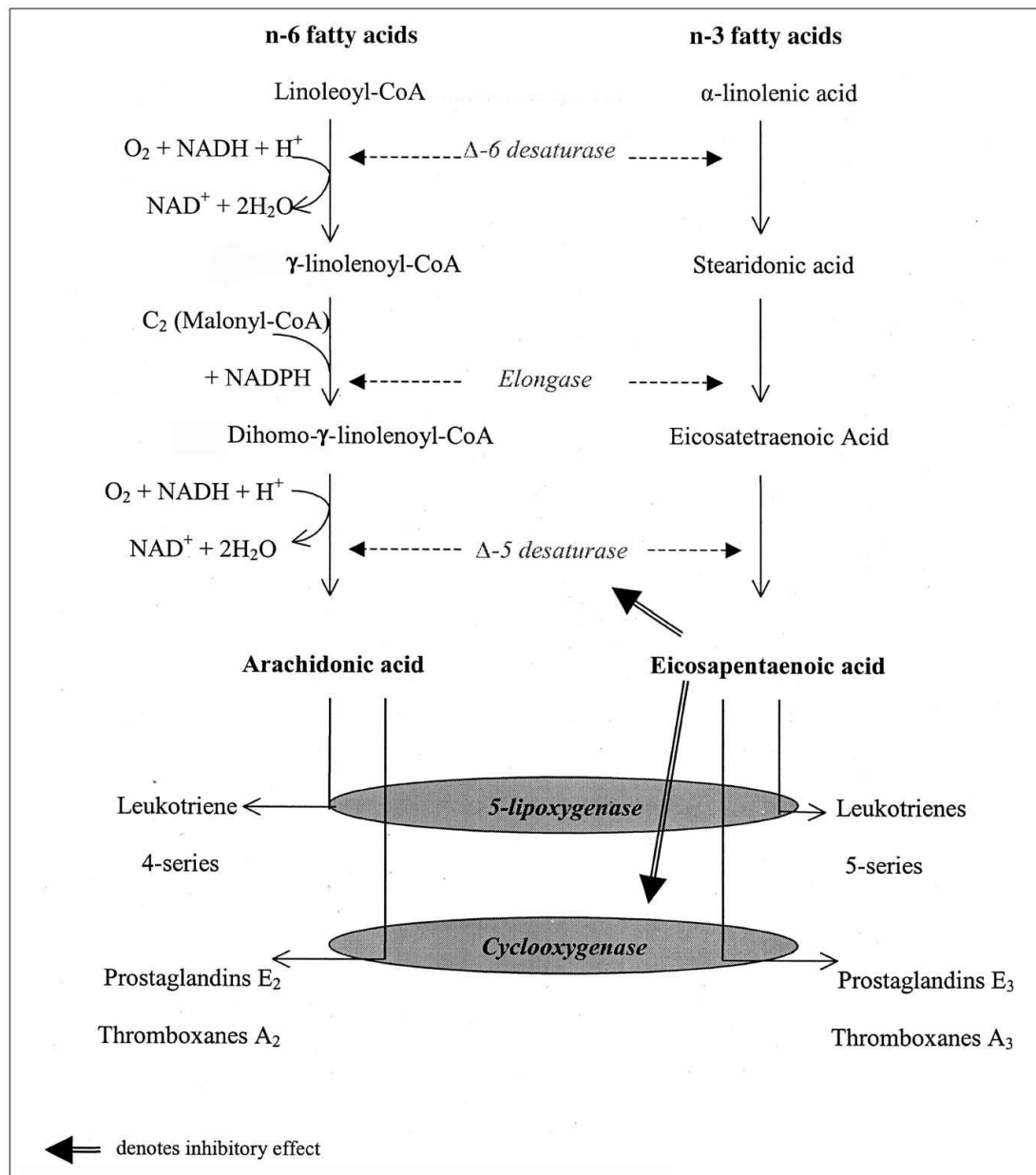


Figure 7- Effect of n-6 and n-3 fatty acids on prostaglandin thromboxane metabolism
(Adapted from Wong 2005).

1.8 TREATMENTS OF ASTHMA

Asthma medications include the use of corticosteroids, anti-inflammatory drugs (mast cell stabilizers), long acting beta-agonists (bronchodilators often used along with an anti-inflammatory drug) (Woisetschlager, Stutz & Ettmayer 2002). Other asthma medications include leukotriene modifiers. These are alternatives to steroids and mast cell stabilizers. New potential therapeutics would ideally minimize the “direct” functionality of IgE by neutralizing IgE (Larche, Robinson & Kay 2003). An

example of this new treatment is Xolair, which blocks IgE. Xolair is used when inhaled steroids for asthma have failed to control asthma symptoms in people with moderate to severe asthma who also have allergies.

Several studies have showed that steroids reduce T-cell activity (Cornejo-Garcia et al. 2007; Duramad, Tager & Holland 2007; Park et al. 2007). However, prolonged use of steroids aggravates unwanted side effects such as slowing of normal growth, development of osteoporosis and affecting control of diabetes, therefore, it is suggested that altering the immune response to asthma by direct or indirect retraction of IgE is a more efficient way for treatment asthma (Bratke et al. 2007; Cornejo-Garcia et al. 2007; Kuiper et al. 2006; Pole et al. 2008). Figure 8 outlines how reducing IgE concentration results in inhibition of mast cell and eosinophil degranulation and cytokine production and decreased mast cell counts.

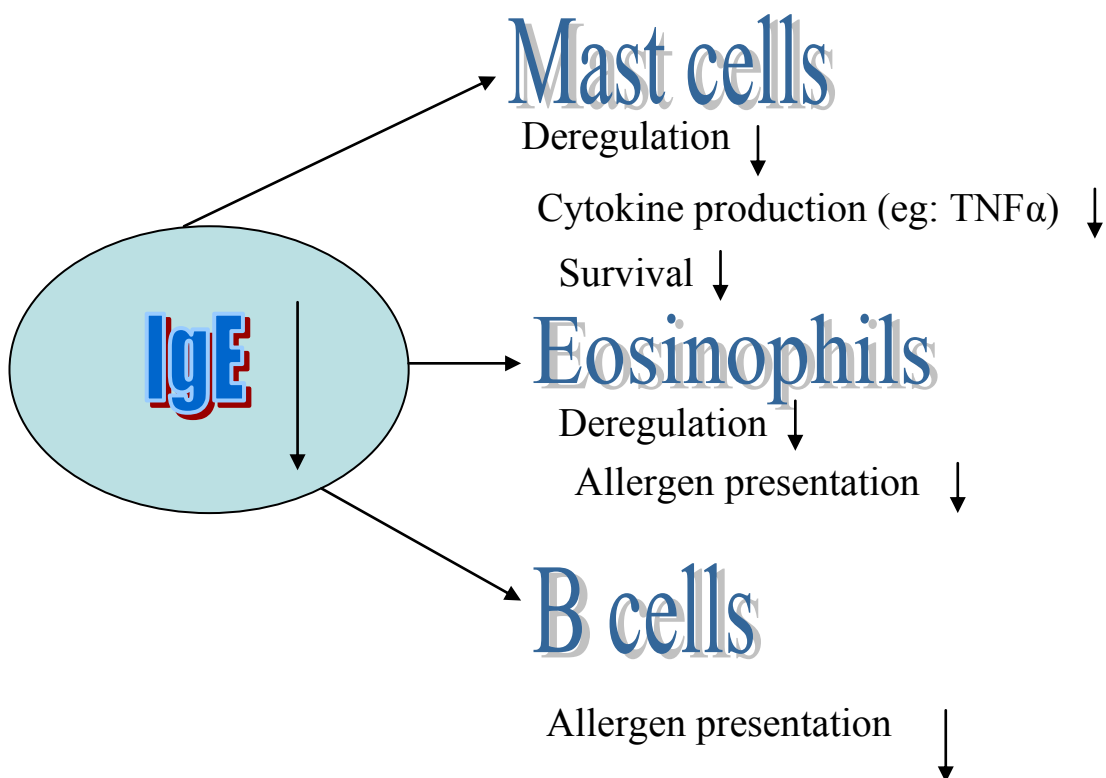


Figure 8- The effects of reduction of IgE on other immune cells
(Compiled by the candidate)

This chapter highlighted the current knowledge on the immunological basis of respiratory diseases with focusing on asthma. It was noted that there are many different immunological components working in synchronization, and any imbalances in their activity may have severe consequences. Most treatment and prevention strategies of asthma are currently focused on allergic/eosinophilic asthma. As discussed earlier (section 1.5), a major proportion of asthma is based on neutrophilic (non-asthmatic) airway inflammation. Eosinophilic asthma is a consequence of the activation of T cells and mast cells in the airway and responds well to steroid treatment. Non-eosinophilic asthma however, is the product of cell mediated and innate immune responses. Therefore, use of steroids is not an effective way of treatment in non- eosinophilic asthmatics. A diet low in antioxidants and high in saturated fat intake may activate innate immune responses in asthma (Wood and Gibson 2009), therefore, there is potential for treatments that target the metabolic pathways. Use of cholesterol lowering drugs such as statins has been shown to reduce innate immune markers such as IL-6, IL-8, and TNF α . Use of antioxidant rich diets such as lycopene has also been shown to modify non-eosinophilic airway inflammation in asthma (Wood and Gibson 2009) .

1.9 SUMMARY

There is an increasing rise in the prevalence of asthma in Western societies over the last two decades compared to developing countries. There are also increasing inconsistencies in the literature over the role of diet in asthma. The aim of this project was to investigate possible associations between nutrients and asthmatic symptoms in 14 year old adolescents enrolled in the Western Australian Pregnancy Cohort (Raine) study.

The hypothesis of this thesis was that consumption of specific antioxidants (vitamins A, C, and E, β -carotene, zinc and fruits rich these nutrients) and n-3 fatty acids will reduce asthmatic symptoms in 14 year old adolescents. There is evidence in the literature suggesting a protective role for these nutrients against asthma (de Luis et al. 2005; Hatch 1995; McKeever & Britton 2004; Soutar, Seaton & Brown 1997,

Chavali, Zhong & Forse 1998, Hennig et al. 1999; Zalewski et al. 2005, Park et al. 2007).

On the other hand, some studies suggest that consumption of n-6 fatty acids will have a detrimental association with asthmatic symptoms (McKeever 2007). Therefore, the objective of this project was to investigate whether consumption of specific antioxidants, n-3 and n-6 fatty acids were associated with asthmatic symptoms in these adolescents.

1.10 HYPOTHESES OF THE STUDY

The hypotheses of the study were that:

1. consumption of specific antioxidants i.e. vitamins A, C, and E, β -carotene, zinc and fruits and vegetables containing these nutrients is lower in asthmatic compared with non-asthmatic 14 year old adolescents.
2. consumption of n-3 fatty acids is lower in asthmatic 14 year old compared with non-asthmatic 14 year old adolescents.
3. consumption of specific antioxidants i.e. vitamins A, C, and E, β -carotene, zinc and fruits containing these nutrients is the lowest in adolescents with asthmatic symptoms at eight and 14 years compared to the non-asthmatic group and those with asthma at eight and 14 years only.
4. consumption of n-3 fatty acids is lowest in adolescents with asthmatic symptoms at eight and 14 years compared to the other groups.

CHAPTER TWO

STUDY METHODS

2.1 INTRODUCTION

This study was based on two longitudinal studies from the West Australian Pregnancy Cohort (Raine) study. The enrolment criteria for the Raine study were gestational age between 16 and 20 weeks, an expectation to deliver at King Edward Memorial Hospital, sufficient proficiency in English to understand the implication of participation and an intention to remain in Western Australia so that follow-up through childhood would be possible. Children have been followed up at birth, one, two, six, eight, ten and 14 years of age during the Raine study. This thesis reports on data collected at eight and 14 years of age.

2.2 THE RAINE STUDY

The Raine study is an ongoing multidisciplinary health research project commenced between August 1989 and March 1992, when approximately 100 women per month were enrolled over a period of 30 months, 2900 women were enrolled (Newnham et al. 1993; Oddy et al. 2004a; Oddy et al. 2000) during their second trimester of pregnancy. Mothers were recruited from the public antenatal clinic at King Edward Memorial Hospital in Perth Western Australia, or surrounding obstetric clinics. This project presents information that has been gathered over fourteen years in the Raine study.

Study One

Study One “the cross-sectional study” was a cross-sectional study of 1531 adolescents at 14 years of age identified as having current asthma (n=167) or no asthma (n=1364). This study examined the first two hypotheses outlined in the previous chapter (section 1.10).

Study Two

Study Two “the case-control study” was a longitudinal analysis from a subset of the overall cross-sectional study. Cases were defined as having current asthma and controls as not having asthma at eight (n=335) and 14 (n=242) years of age (Oddy et al. 2004a). The third and fourth hypotheses outlined in the previous chapter (section 1.10) were examined by Study Two.

The children were selected as cases and controls at eight years of age as follows in an earlier study (Oddy et al. 2004a):

Case ascertainment

During 1999 when the children were eight years of age, all cases of current asthma were identified at six years. The diagnosis of asthma was confirmed from the six year assessment by diagnosis of asthma by a doctor and wheeze in the last year or using preventer or reliever medication (n=147). Mothers were telephoned, invited to participate and the child’s asthma status confirmed. Case children whose mother consented to participate by telephone were sent dietary questionnaires. During control ascertainment, parent reported current asthma at age eight but not at age six resulted in the child designated as a case (n=19). If a parent reported that an identified case from the six year follow-up no longer had asthma they were excluded from the study.

Control ascertainment

Controls were children with no asthma diagnosis, no current wheeze and not taking asthma medication at either six or at eight years of age. At the same time as case ascertainment, controls were selected (one per case) on gender and age (birth date within one month). One control was selected from the potential controls for each case (usually about 12) and the child’s absence of asthma was confirmed through

personal contact with parent. Controls whose parent consented to participate were sent dietary questionnaires.

In this thesis, the role of risk factors for asthma and atopy in children was examined with specific attention given to the roles of antioxidant and n-3 fatty acids for both studies.

Administration of the Raine study and the questionnaires was conducted by the project coordinator, research nurses and research assistants employed on the large overall cohort study. Dietary data were collected using the CSIRO food frequency questionnaire (Baghurst & Record 1983). The analytical methods for Study One and Two were developed by Dr Wendy Oddy, Professor Nick deKlerk, Peter Jacoby and the DrPH candidate during the period of this study (2006-2007).

2.3 POPULATION GROUP FOR STUDY ONE AND STUDY TWO

At birth 2868 infants from 2900 women were available for follow up. Parents (n=2900) of the children completed a questionnaire about their own respiratory illnesses, smoking behaviour and general health conditions at the time of enrolment. Detailed data on each infant including gestational age, birth weight and gender were collected at birth; however, these data were not used in the analysis for this thesis.

Informed consent from parents was also obtained at the birth of their children and at each subsequent follow up for their child's participation in the study and follow-up assessments.

2.4 DATA COLLECTION FOR STUDY ONE: CROSS SECTIONAL STUDY OF 14 YEAR OLD ADOLESCENTS

Study One investigated if there was an association between the current intake of antioxidants and n-3 fatty acids with asthmatic traits in 14 year olds using questionnaire data, physical assessment and red blood cell analysis.

2.4.1 Food Frequency Questionnaire (FFQ)

Dietary data were collected by the CSIRO food frequency questionnaire (Baghurst & Record 1983) (See Appendix 9). The FFQ was validated in adults (Yearsley, Last & Ward 1999) and has previously been validated and applied for fish intake against serum fatty acids samples in the eight year follow-up in children in the Raine study (Oddy et al. 2004a). These questionnaires were very comprehensive and contained questions on a variety of 212 foods and nutrients.

The parents, in discussion with the children, were asked to complete the dietary questionnaire to describe their child's usual eating patterns over the past year as well as seasonal variations. By using the number of times a food was eaten per day, week, or month, the best total estimate of the child's usual diet was made. Seasonal differences were measured by asking how often foods were eaten during both summer and winter. (See Appendix 4)

This questionnaire was posted to all the participants and completed questionnaires were then reviewed by nurses to ensure that all the sections had been completed. Follow up calls were made by nurses when necessary for completion of questionnaires.

Completed questionnaires were posted to CSIRO for data entry by skilled data entry personnel. Completed data sets were returned to Dr Wendy Oddy as SPSS data files.

2.4.2 Dietary and nutrient exposures

The average daily intake of the dietary antioxidant nutrients were estimated from the fruit and vegetable consumption in FFQ. The antioxidants of interest were: vitamin A ($\mu\text{g/day}$), vitamin C (mg/day), vitamin E (mg/day), magnesium (mg/day) and zinc (mg/day). Foods high in antioxidant nutrients included fruits (berry, citrus, stone, pome [apples and pears] and tropical fruits) (g/day), vegetables (carrots, turnip, broad beans, green beans, capsicum, cabbages, green peas, Brussels sprouts, spinach, broccoli, cauliflower, pumpkins, sweet corn, and zucchini)(g/day). Average daily intake of the total n-3 and n-6 were also estimated from the FFQ (g/day).

2.4.3 Red blood cell fatty acids

The possible association of the red blood cell fatty acids (α -linolenic acid, parinaric acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, linoleic acid, arachidonic acid and docosatetraenoic acid) (mmol/L), total n-3 (mmol/L), total n-6 (mmol/L), and the ratio of n6:n3 with the asthmatic outcome of interest were also investigated.

A fasting red blood cell sample was collected from adolescents at the 14 year follow-up ($n=1329$). A phlebotomist attended each study child first thing in the morning to collect fasting blood samples. Fatty acid analyses of red blood cells were carried out by Dr Trevor Mori of School of Medicine & Pharmacology Royal Perth Hospital, as previously described (Mori et al. 2000). Briefly, total lipids were extracted with chloroform: methanol (2:1) and fatty acid methyl esters were prepared by treatment of extracts with 4% H_2SO_4 in methanol at 90°C for 20 minutes and analysed by gas liquid chromatography using a Hewlett-Packard model 5980A gas chromatograph. The column was a BPX70 (25m x 0.32mm, 0.25 μm film thickness) (SGE, Ringwood, Victoria, Australia) with a temperature programmed from 150°C to 210°C at 4°C/min and using N_2 as the carrier gas at a split ratio of 30:1. Peaks were identified by comparison with a known standard mixture. The fatty acids were expressed as a percentage of the weight of the total fatty acids measured (C_{14} to C_{22}) (Mori et al. 2000). Percentage of the weight of the total fatty acids was quantified

using an internal standard to provide the concentration (mmol/L). This facilitated the comparison between the mean level of fatty acids in the cohort and that of the healthy population.

Fatty acids of interest for this study were: n-3: α -linolenic acid (18:3n3), parinaric acid (18:4n3), eicosapentaenoic acid (20:5n3), docosapentaenoic acid (22:5n3), docosahexaenoic acid (22:6n3) and n-6: linoleic acid (18:2n6), arachidonic acid (20:4n6) and docosatetraenoic acid (22:4n6).

The red blood cell fatty acid data were used to investigate the possible association of individual fatty acids, total n-6, total n-3 and the ratio of n6:n3 fatty acids on the outcome of interest (described below) at 14 years of age in Study One. The phlebotomist was employed on the study to increase the fasting blood collection response rate.

2.4.4 Physical assessment:

Physical assessments included Skin Prick Test (SPT) and test for bronchial hyperresponsiveness (BHR). These tests are described in sections 2.5.4 and 2.5.5.

2.4.5 Respiratory questionnaire

Asthmatic outcomes were measured at 14 years of age using a respiratory questionnaire (See Appendix 3). The questions asked in the respiratory questionnaires were based on the American Thoracic Society questionnaire and published elsewhere (Oddy et al. 2004a). This questionnaire included questions that captured the outcomes of wheeze frequency and asthma severity and other asthmatic symptoms. The respiratory questionnaire was based on the International Study of Asthma and Allergies in Childhood (ISSAC) study questionnaire (Tabak et al. 2005) and other related respiratory questionnaires (Parrish et al. 2003).

The definitions of the asthmatic outcomes used in this study are described in following sections.

2.5 ASTHMATIC SYMPTOMS: OUTCOMES FOR STUDY ONE (THE CROSS-SECTIONAL STUDY AT 14 YEARS)

The outcomes of interest for Study One included current asthma, current wheeze, bronchial hyperresponsiveness (BHR), asthma severity and atopy.

2.5.1 Current asthma

Current asthma was defined by a positive response to the following question:
Was asthma diagnosed by a doctor within the past 12 months?

2.5.2 Current wheeze and number of wheezing attacks

Current wheeze was defined as having any wheezing episodes reported in the questionnaire in the past 12 months (Joseph-Bowen et al. 2004). The adolescents were also divided according to The International Study of Asthma and Allergies in Childhood (ISAAC), into the following 4 groups based on the number of wheezing episodes within the last 12 months: none, 1-2, 3-12 and more than 12 (Pearce et al. 2007).

2.5.3 Asthma severity

Severity of asthma was defined as mild, moderate, or severe on the basis of the main symptoms of episodes of wheezing per year (Joseph-Bowen et al. 2004). If 1 to 2 episodes of wheeze were reported in the past 12 months, then asthma was considered to be mild. Asthma was considered to be moderate, if 3 to 12 wheezing episodes and on average not more than one occasion of nocturnal waking per week caused by wheeze had been reported during the past 12 months. Severe asthma was defined as having at least one of the following symptoms: more than 12 episodes of wheeze,

one or more episodes of acute asthma limiting speech, or more than one occasion of nocturnal waking per week (on average) throughout the past 12 months.

2.5.4 Atopy

The skin-prick test (SPT) was performed to determine the presence of atopic dermatitis in 14 year old children. This test was carried out by pricking through a drop of allergen extract (house dust mite, grasses, cat, dog, moulds, egg protein and peanut) placed on the surface of the arm (Clausen et al. 2008). The test showed that a level of anti-bodies were/or were not present which may be causing allergic symptoms. If the test was positive it resulted in the appearance of a small, itchy swelling and a reddening of the skin one or more positive SPT of equal or more than 3 mm in diameter was recorded as atopy positive (Clausen et al. 2008).

2.5.5 BHR

The degree of bronchial hyperresponsiveness (BHR) was tested according to the American Thoracic Society (ATS) using the challenge as described below (Joseph-Bowen et al. 2004).

Inhalations of saline and methacholine were delivered using a dosimeter to ensure controlled doses were delivered. The children who completed this protocol (n=1479) initially received an inhalation of normal saline, followed by increasing doses of methacholine. Lung function Forced Expiratory Volume (FEV₁) was measured by the research nurse 30 seconds and 90 seconds after each inhalation.

The next dose of methacholine was delivered provided the FEV₁ had not fallen by 20% of the post-saline value. Doubling doses of Methacholine from 0.03 to 7.8 µM were delivered. The test was finished when the FEV₁ had fallen by 20% of the post saline value or the highest dose was delivered. A dose-response curve was constructed and the dose that caused a 20% fall in FEV₁ (PD₂₀ < 7.8 µM) was recorded as a positive BHR (Joseph-Bowen et al. 2004).

2.6 DATA COLLECTION FOR STUDY TWO: THE CASE-CONTROL STUDY

Study Two was a longitudinal analysis from a subset of the overall prospective cohort study. Data were used from both the eight and 14 year follow-up of the cohort. Doctor diagnosed asthma cases were selected from eight-year-old children in a previous study (Oddy et al. 2004b). These cases, identified at eight years were combined with cases of current asthma at 14 years of age.

A new variable of history of asthma was created with four sub-groups (please see Table 24). Group 1: had no asthma at either ages of eight or 14, group 2: had asthma only at the age of eight, group 3: had asthma only at the age of 14 and group 4 had asthma at both ages of eight and 14 years.

The data from the food frequency questionnaire (FFQ) at 14 years of age was used to investigate the intake of the nutrient of interest for each group.

The number of adolescents participating at 14 year follow-up was reduced compared with participation at eight years, due to several factors including loss to follow-up, withdrawal from the study or non-participation in this phase of the study.

Dietary intake of fatty acids (n-3: α -linolenic acid, parinaric acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, n-6: linoleic acid, arachidonic acid, docosatetraenoic acid, total n-3, total n-6 and the ratio of n6:n3) intake was estimated through CSIRO data bases for fatty acids in these foods, as described in section 2.3.2 (fish, cereals, legumes, red meat and chicken).

The information gathered from the FFQ (fatty acids, fruits, vegetables and antioxidants) data were used for analyses of the previously selected cases and controls at eight years of age (Oddy et al. 2004a) and for assessing their dietary status six years later at 14 years of age.

2.6.1 Observer bias

The nurses conducting the physical assessments (as described in 2.3.4) were blinded to the information contained in the questionnaires when performing these assessments to eliminate observer bias.

2.7 ASTHMATIC OUTCOMES FOR STUDY TWO: THE CASE-CONTROL STUDY

The outcome of interest for the case-control study was asthma history which was based on doctor diagnosed asthma. Combining doctor diagnosed asthma at the age of eight as one variable with current asthma (at 14 years of age) or not as another variable, a new variable of history of asthma was created with 4 sub-groups. Group 1: had no asthma at either ages of eight or 14, group 2: had asthma only at the age of eight, group 3: had asthma only at the age of 14 and group 4 had asthma at both ages of eight and 14 years.

2.8 STATISTICAL ANALYSIS

2.8.1 Study One

Consumption estimates of various antioxidants (listed in section 2.4.2), were obtained from the food frequency questionnaires from all participants.

The average daily intake of dietary antioxidant, fruits, vegetables and the mean red blood cells fatty acid content of total n-3, total n-6, n6:n3 ratio and mean of individual n-3 fatty acids; [α -linolenic acid (18:3n3), parinaric acid (18:4n3), eicosapentaenoic acid (20:5n3), docosapentaenoic acid (22:5n3), docosahexaenoic acid (22:6n3)] and n-6 [linoleic acid (18:2n6), arachidonic acid (20:4n6), docosatetraenoic acid (22:4n6)] fatty acids were compared in association with asthma severity and the number of times wheezed over the last 12 months by using one way ANOVA. The Fisher's Least Significance Difference (LSD) test was

applied to compare fatty acid content between the two groups' means after ANOVA was performed.

All the children in Study One were divided into five groups (quintiles) according to the fatty acids content in their red blood cells (RBC). Quintiles were used to make the results comparable to groupings already identified in the literature (Farchi et al. 2003; McKeever 2007; Varraso et al. 2007). The groups spanned from the lowest to the highest fatty acid content of interest. It was then determined if there were any significant differences between the respective fatty acid content between individual groups (Pearson chi square) as well as any trends across all groups (trend p-value) with any of the outcomes of interest (current asthma, current wheeze, BHR and atopy) for the individual fatty acids. Two-tailed tests of significance were used with $\alpha=0.05$. Depending on the outcome of interest, multinomial or binary logistic regression was performed to control for potential confounding effects of age, sex, BMI and total daily energy intake. Odds Ratio (OR) and 95% confidence interval (95% CI) were computed to estimate the degree of association.

2.8.2 Study Two

As in Study One, dietary antioxidant data were collected using FFQ and fatty acid intake was estimated as there was no RBC data available for eight year old children. This method has previously been shown to be valid (Oddy et al. 2004b). Dietary antioxidants and fatty acid data were assessed for normality and log-transformed if necessary. ANOVA was performed for different antioxidants and fruits and different fatty acid intake with a history of asthma to make comparisons between the means of these nutrients and presence or absence of asthma history. The mean for each nutrient intake was compared between the entire four groups (group 1: no history of asthma, group 2: asthma at eight, group 3: asthma at 14 and group 4: asthma at eight and 14). The Fisher's Least Significance Difference (LSD) test as a post hoc comparison procedure was also performed when ANOVA resulted in a significant F test. One way analysis of variance (ANOVA) allowed comparisons of food and nutrient intakes between the groups. Fatty acids that were significant in these analyses ($p<0.05$) were included in analysis of covariance (ANCOVA) to determine

whether they made an independent contribution on the outcome of interest (history of asthma) after adjustment for age, sex, BMI and total energy intake. Bonferroni post hoc tests were applied to control for false positive results.

2.8.2.1 Statistical power for Study Two

Subjects were divided into groups based on presence or absence of asthma. Power calculations were performed based on the actual size in each group. The power calculation showed that a sample size of $n=123$ achieves 91.4% power to detect a difference of 0.3 between the means with a significance level of 0.05 using a 2-sided paired samples. When $n=68$ statistical power =69.6%, $n=6$ power=11.4% and when $n=45$ power=52.1%.

2.9 ETHICS APPROVAL FOR STUDY ONE AND TWO

Ethics approval to conduct the analysis for the studies One and Two that form this thesis were obtained at candidacy from Curtin University Human Ethics Committee (Protocol Approval Number: SP-019-2007) (See Appendix 1).

Parents were informed prior to agreeing to participate, that the study would be ongoing over a number of years and would involve the investigation of child health outcomes. The Human Ethics Committee at King Edward Memorial Hospital for Children, Perth, Western Australia had approved the protocol for the Western Australian Pregnancy Cohort Study (See Appendix 2). At the time of enrolment and at each follow-up mothers consented to participate in the study (See Appendix 6).

The work of this thesis represents a collaboration between the School of Public Health at Curtin University and the Telethon Institute for Child Health Research.

Ethics approval was obtained from parents for the eight year follow-up and also from the teenage study participants for the 14 year follow-up. Information sheets were provided for parents and teens and a consent form was signed by each participant for

clinical assessments. Parents and teens were informed that their refusal to participate in any aspect of the study would not affect future care in the health system. Parents were advised that any tests could be abandoned if the adolescent declined to cooperate.

CHAPTER THREE

STUDY ONE RESULTS

Results from Study One are reported in this chapter. The results for Study Two are reported in Chapter Four. Study One was conducted to investigate if there was a significant association between the intake of antioxidants and n-3 fatty acids with asthma manifestations (wheeze, asthma, atopy and bronchial hyperresponsiveness) by using data from food frequency questionnaires (FFQ). Also, for this study, red blood cell fatty acid data were used to investigate the possible association of individual fatty acids, total n-3, total n-6 and the ratio of n6:n3 fatty acids on the asthma manifestation.

3.1 THE RESULTS OF STUDY ONE:

Study One was a cross-sectional study that included adolescents at age 14 participating in the follow-up identified as having current asthma (n=167) or no asthma (n=1364) in this cohort.

3.1.1 Cohort characteristics

Characteristics of the cohort under investigation are summarized in Table 1. The participants were 50.8% male and 49.2% female. The main outcomes of the study, separate measurements of wheeze, asthma, atopy and bronchial hyperresponsiveness, were found to be positive in between 10.9% to 44.5% of adolescents. One hundred and sixty seven adolescents (10.9%) of the cohort had asthma. Among those with current asthma, different levels of severity were observed (mild, moderate and severe) with moderate asthma being most prevalent (4.4%) followed by mild (4.0%) and severe asthma (2.5%). Two hundred and eighteen adolescents of the total cohort (14.2%) were reported to have current wheeze. Different allergy outcomes were observed; bronchial hyperresponsiveness (17.9%), allergic conjunctivitis (27.3%),

itchy eyes (25.4%), eczema (10.7%), hay fever (37.3%), and atopy (44.5%) for this population. The most common allergy outcome was atopy. The least common allergy outcome was eczema (Table 1)

Table 1: Characteristics of subjects in Study One

| Outcome at 14 years | Frequency N=1531 | Percentage |
|--|-----------------------------|-------------------|
| Current wheeze | 218 | 14.2 |
| *Number of wheeze attacks in the last 12 months | | |
| 0 | 1314 | 85.9 |
| 1-2 | 129 | 8.4 |
| 3-12 | 67 | 4.4 |
| >12 | 20 | 1.3 |
| Current asthma | 167 | 10.9 |
| Asthma severity | | |
| None | 1364 | 89.1 |
| Mild | 62 | 4.0 |
| Moderate | 67 | 4.4 |
| Severe | 38 | 2.5 |
| Bronchial Hyperresponsiveness | 274 | 17.9 |
| Allergic conjunctivitis | 418 | 27.3 |
| Itchy eyes | 389 | 25.4 |
| Eczema | 164 | 10.7 |
| Hayfever | 572 | 37.3 |
| Atopy | 682 | 44.5 |
| Child characteristics | | |
| Gender | | |
| Male | 778 | 50.8 |
| Female | 773 | 49.2 |

*Total number of participants who answered the number of wheeze attacks was 216 as 2 participants did not complete this section in the questionnaire.

3.1.2 Red blood cell membrane fatty acid content.

Red blood cell (RBC) membrane fatty acid content was analysed from 1304 participants, Table 2 describes mean and range of the RBC membrane fatty acid content. Individual mean RBC membrane fatty acid content was diverse among individuals. The highest range was observed for arachidonic acid (20:4n6) with a range of (2.93-20.17 mmol/L) and the lowest range was observed in eicosapentaenoic acid (20:5n3) with a range of (0.0-1.91 mmol/L).

The level of parinaric acid (18:4n3), docosapentaenoic acid (22:5n3) and linoleic acid (18:2n6) were above the levels of the healthy population, while the level of α -linolenic acid (18:3n3), arachidonic acid (20:4n6) and docosatetraenoic acid (22:4n6) were below the average of the healthy population range. Eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3) levels were very similar to the healthy population levels (Table 2).

Table 2: Red blood cell fatty acid content for the cohort (n=1304) and a healthy population.

| Nutrient | Cohort mean (mmol/L) \pm SD | Cohort range (mmol/L) | *Healthy population mean (mmol/L) \pm SD |
|--|---|----------------------------------|--|
| 18:3n3 Alpha-linolenic acid (ALA) | 0.22 \pm 0.25 | 0.0-3.36 | 0.30 \pm 0.02 |
| 18:4n3 Parinaric acid | 0.54 \pm 0.45 | 0.0-8.54 | 0.31 \pm 0.001 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.71 \pm 0.21 | 0.0-1.91 | 0.66 \pm 0.04 |
| 22:5n3 Docosapentaenoic acid (DPA) | 4.33 \pm 2.15 | 0.29-11.17 | 2.93 \pm 0.08 |
| 22:6n3 Docosahexaenoic acid (DHA) | 4.29 \pm 1.07 | 0.69-9.91 | 4.70 \pm 0.18 |
| 18:2n6 Linoleic acid | 10.06 \pm 1.21 | 3.12-14.61 | 5.94 \pm 0.10 |
| 20:4n6 Arachidonic acid (AA) | 13.46 \pm 2.17 | 2.93-20.17 | 19.77 \pm 0.29 |
| 22:4n6 Docosatetraenoic acid | 1.61 \pm 2.02 | 0.0-8.21 | 6.45 \pm 0.12 |

*(Harris et al. 2005).

3.1.3 Daily nutrient/antioxidant and energy intake

Daily nutrient/antioxidant intake for Study One subjects who responded to the food frequency questionnaire (n=1531) is shown in Table 3. The level of recommended daily intake for this population group is also described in Table 4 (Baghurst et al. 2005; Bhagavan 2002).

The smallest range of daily nutrients consumed among this population was observed for n-3 fatty acids ranging from 0.27-6.03 g/day (Table 3).

The daily intake of vitamin C was very diverse among the cohort (191.8 ± 116.39 mg/day) and was considerably higher than the recommended daily intake of 45 mg/day. The mean daily intake of vitamin E was 8.78 ± 4.61 mg/day which was below the recommended daily intake of 11 mg/day. The recorded average daily intake for zinc was 12.88 ± 4.42 mg/day and this was very similar to the recommended daily intake of 10-15 mg/day.

The mean daily intake of n-3 was within the recommended daily intake. However, the average daily intake of n-6 was nearly 5 times more than the recommended daily amount of 2.4-5.6 g/day. The ratio of n6:n3 was consequently higher (9.45 ± 4.24) compared with the average recommended ratio of 2-4 (Katrine Baghurst et al. 2005; Bhagavan 2002).

Table 3: Daily nutrient/antioxidant intake for the cohort who responded to the food frequency questionnaire (n=1531). Recommended daily intake (RDI) is also described for this population group.

| Food/Nutrient | Mean \pm SD | Range | *Recommended daily intake (RDI) |
|----------------------------------|---------------------------------|-----------------|--|
| Vitamin A (μ g/day) | 1240.05 \pm 656.99 | 199.89- 8904.26 | 600-900 |
| Vitamin C (mg/day) | 191.8 \pm 116.39 | 6.79- 1047.2 | 45 |
| Vitamin E (mg/day) | 8.78 \pm 4.61 | 1.26-41.01 | 11 |
| β -carotene (μ g/day) | 4324.84 \pm 2220.97 | 315.38-20588.47 | 6000-15000 |
| Zinc (mg/day) | 12.88 \pm 4.42 | 2.85-36.87 | 10-15 |
| Magnesium (mg/day) | 319.49 \pm 113.57 | 89.2-914.07 | 240 |
| n-3 (g/day) | 1.30 \pm 0.66 | 0.27-6.03 | 1.2-1.4 |
| n-6 (g/day) | 11.67 \pm 6.42 | 1.56-77.9 | 2.4-5.6 |
| n6:n3 | 9.45 \pm 4.24 | 1.84-101.2 | 2-4 |

(Katrine Baghurst et al. 2005; Bhagavan 2002).

The average daily energy intake for girls in our study was 2327.78 Kcal \pm 736.78 SD with a mean weight of 57.4 kg \pm 13.67 SD and an average height of 1.63 m \pm 0.10 SD. This is significantly ($p < 0.05$) more than the average recommended daily energy intake of 2192 Kcal for a reference weight of 49.4 kg and reference height of 1.60 m for 14 year girls (Nutrient Reference Values for Australia and New Zealand (Baghurst et al. 2005; Bhagavan 2002)). The average daily energy intake for boys in our study was 2378.33 Kcal \pm 836.46 SD with a mean weight of 57.93kg \pm 12.89 SD and an average height of 1.64 m \pm 0.09 SD. This was significantly ($p < 0.05$) less than the 2531 Kcal for a reference weight of 51 kg and reference height of 1.64 m average recommended daily energy intake for 14 year boys (Table 4).

There was no significant difference in total energy intake between genders in our study ($p = 0.209$).

The average daily total fat intake for our cohort was 92.79g \pm 38.89 SD which is 43% higher than the recommended daily intake of 65 g/day. The average intake of saturated fat was 40.91 g \pm 17.72 SD that is twice the amount of RDI (20 g/day) (Katrine Baghurst et al. 2005; Bhagavan 2002). There was a significant difference in total fat intake between males (94.03 g/day \pm 38.09 SD) and females (92.25 g/day \pm 33.82 SD) ($p = 0.004$).

Table 4: Daily fat/energy intake for the cohort who responded to the food frequency questionnaire (n=1531). Recommended daily intake (RDI) is also described for this population group.

| Food/Nutrient | Mean \pm SD | Range | **Recommended daily intake (RDI) |
|----------------------------|---------------------------------|--------------------|---|
| Energy (Kcal/day) Girls | 2327.78 \pm 736.78 | 645.75-5718.92 | 2192 |
| Energy (Kcal/day) Boys | 2378.33 \pm 836.46 | 790.08-6682.45 | 2531 |
| *Total fat (g/day) | 92.79 \pm 38.89 | 17.63 \pm 267.65 | 65 |
| *Saturated fat (g/day) | 40.91 \pm 17.72 | 7.89 \pm 138.28 | 20 |

* Average for cohort ** (Baghurst et al. 2005; Bhagavan 2002).

3.1.4 Association between daily nutrient/antioxidant and food intake and allergy outcomes

Differences among average daily nutrients and antioxidant intake were analysed using independent samples T-test for different allergic outcomes of wheeze, asthma, atopy and bronchial hyperresponsiveness (BHR) (Tables 5-12).

3.1.4.1 Association between daily nutrient/antioxidant and food intake and wheezing

The association between daily nutrient/antioxidant and food intake and wheezing is described in Table 5. There was no significant difference between mean dietary intakes of vitamin E between wheezers (those adolescents with more than one wheeze attack in the last 12 months) and non-wheezers. After adjusting for confounders (age, sex, BMI and total energy intake), none of the foods and nutrients of interest were shown to be a significant risk for wheezing (Table 6).

Table 5: Daily nutrient/antioxidant intake for wheezers and non-wheezers, mean \pm SEM using independent samples T-test (n=1531).

| Food/Nutrient | Wheezers Mean (SEM) N=218 | Non-wheezers Mean (SEM) N=1313 | <i>P-value</i> |
|-----------------------------------|--|---|-----------------------|
| Vitamin A (μ g/day) | 1304.92 (63.79) | 1228.97 (18.10) | 0.253 |
| *Vitamin C (mg/day) | 2.19 (0.02) | 2.20 (0.007) | 0.425 |
| *Vitamin E (mg/day) | 0.91 (0.016) | 0.88 (0.006) | 0.096 |
| Zinc (mg/day) | 13.17 (0.34) | 12.80 (0.12) | 0.280 |
| Magnesium (mg/day) | 320.56 (8.55) | 317.26 (3.24) | 0.707 |
| β -carotene (μ g/day) | 2694.12 (255.31) | 2519.59 (98.52) | 0.498 |
| *N-3 (g/day) | 0.08 (0.014) | 0.06 (0.006) | 0.296 |
| *N-6 (g/day) | 1.04 (0.016) | 1.01 (0.007) | 0.086 |
| n6:n3 | 9.91 (0.29) | 9.44 (0.11) | 0.094 |
| *Steamed /grilled fish (g/day) | 1.71 (0.032) | 1.68 (0.013) | 0.418 |
| *Sea food (g/day) | 0.71 (0.04) | 0.69 (0.02) | 0.647 |
| Pome fruits | 1.81 (0.03) | 1.79 (0.01) | 0.529 |
| Berry fruits | 7.55 (1.46) | 7.99 (0.4) | 0.696 |
| *Citrus fruits | 1.43 (0.04) | 1.40 (0.02) | 0.499 |
| *Stone fruits | 1.24 (0.05) | 1.29 (0.02) | 0.269 |
| Tropical fruits | 51.88 (3.99) | 58.52 (2.03) | 0.213 |
| Dried/preserved fruit | 4.27 (0.81) | 4.75 (0.39) | 0.645 |
| *Fresh vegetables (g/day) | 1.88 (0.017) | 1.85 (0.009) | 0.242 |

(*logarithmic value of nutrients).

Table 6: Adjusted and unadjusted odds ratio (OR) for the risk of current wheeze for different foods/nutrients.

| Food/Nutrient | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|--------------------------|-----------------|---------------|---------------------|---------------|
| Vitamin A | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| Vitamin C | 1.000 | 0.998,1.001 | 0.998 | 0.996,1.000 |
| Vitamin E | 1.027 | 0.994,1.060 | 1.024 | 0.969,1.083 |
| Zinc | 1.019 | 0.985,1.054 | 1.025 | 0.939,1.118 |
| Magnesium | 1.000 | 0.999,1.002 | 1.000 | 0.997,1.002 |
| β-carotene | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| N-3 | 1.051 | 0.989,1.117 | 1.031 | 0.942,1.129 |
| N-6 | 0.975 | 0.935,1.016 | 0.988 | 0.931,1.048 |
| n6:n3 | 0.876 | 0.752,1.020 | 0.930 | 0.749,1.156 |
| Steamed /grilled fish | 1.000 | 0.997,1.004 | 0.998 | 0.994,1.003 |
| Sea food | 1.004 | 0.962,1.048 | 0.985 | 0.922,1.053 |
| Pome fruits | 1.000 | 0.998,1.002 | 0.999 | 0.996,1.001 |
| Berry fruits | 0.998 | 0.987,1.009 | 0.999 | 0.986,1.012 |
| Citrus fruits | 1.000 | 0.997,1.003 | 0.995 | 0.990,1.000 |
| Stone fruits | 0.998 | 0.994,1.003 | 0.997 | 0.991,1.003 |
| Tropical fruits | 0.998 | 0.996,1.001 | 0.998 | 0.995,1.002 |
| Dried/preserved fruit | 0.997 | 0.985,1.010 | 0.996 | 0.981,1.012 |
| Fresh vegetables | 1.000 | 0.997,1.002 | 0.998 | 0.994,1.002 |

*Odds ratio adjusted for age, sex, BMI and total energy intake.

3.1.4.2 Association between daily nutrient/antioxidant and food intake and asthma

The association between daily nutrient/antioxidant and food intake and asthma is described in Table 7. There were no significant differences between mean dietary intakes of vitamins A,E,C, magnesium, zinc β -carotene, n-3, n-6 fatty acids, steamed/grilled fish, sea foods and different fruits and vegetables between those who have doctor diagnosed asthma and those who didn't have asthma.

The mean intake of vitamin C was slightly lower for asthmatic compared with non-asthmatic adolescents (2.17 compared with 2.20); however this difference was not statistically significant.

After adjusting for confounders (age, sex, BMI and total energy intake), increased intake of vitamin C showed a protective effect on asthma (adjusted OR= 0.997, 95% CI= 0.994–0.999). For every 1 mg/day increase in intake of vitamin C there was a 0.3% reduction in the risk of developing asthma (Table 8).

Table 7: Daily nutrient/antioxidant intake for asthma and non-asthma cases, mean \pm SEM using independent samples T-test (n=1531)

| Food/Nutrient | Asthma Mean (SEM) N=167 | Non-Asthma Mean (SEM) N=1364 | <i>P-value</i> |
|---|--|---|-----------------------|
| Vitamin A (μ g/day) | 1274.00 (74.84) | 1235.34 (17.97) | 0.507 |
| *Vitamin C (mg/day) | 2.17 (0.023) | 2.20 (0.007) | 0.174 |
| Vitamin E (mg/day) | 9.16 (0.405) | 8.74 (0.126) | 0.284 |
| Zinc (mg/day) | 12.93 (0.386) | 12.84 (0.123) | 0.826 |
| *Magnesium(mg/day) | 2.47 (0.013) | 2.48 (0.004) | 0.575 |
| * β -carotene (μ g/day) | 3.26 (0.049) | 3.24 (0.018) | 0.642 |
| N-3 (g/day) | 1.32 (0.018) | 1.29 (0.057) | 0.640 |
| *N-6 (g/day) | 1.03 (0.018) | 1.01 (0.006) | 0.451 |
| n6:n3 | 9.85 (0.345) | 9.46 (0.099) | 0.208 |
| *Steamed /grilled fish (g/day) | 1.71 (0.037) | 1.68 (0.013) | 0.555 |
| *Sea food (g/day) | 0.73 (0.046) | 0.69 (0.015) | 0.356 |
| *Pome fruits | 1.83 (0.039) | 1.79 (0.013) | 0.339 |
| Berry fruits | 6.81 (1.167) | 8.07 (0.425) | 0.334 |
| *Citrus fruits | 1.44 (0.042) | 1.40 (0.015) | 0.449 |
| *Stone fruits | 1.24 (0.052) | 1.29 (0.017) | 0.335 |
| Tropical fruits | 52.67 (4.79) | 58.19 (1.97) | 0.356 |
| *Fruits combined (Pome, berry, citrus, stone) | 2.03 (0.039) | 2.02 (0.013) | 0.791 |
| Mixture of 2 or more group of fruits | 18.89 (2.33) | 20.31 (0.789) | 0.557 |
| Dried/preserved fruit | 3.88 (0.899) | 4.78 (0.386) | 0.439 |
| Fresh vegetables (g/day) | 83.80 (4.388) | 87.33 (1.608) | 0.473 |

(*logarithmic value of nutrients).

Table 8: Adjusted and unadjusted odds ratio (OR) for the risk of current asthma for different foods/nutrients.

| Food/Nutrient | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|-----------------------|-----------------|---------------|---------------------|--------------------|
| Vitamin A | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.001 |
| Vitamin C | 0.999 | 0.998,1.001 | **0.997 | 0.994,0.999 |
| Vitamin E | 1.020 | 0.984,1.058 | 1.044 | 0.981,1.112 |
| Zinc | 1.004 | 0.966,1.044 | 1.025 | 0.928,1.132 |
| Magnesium | 1.000 | 0.998,1.002 | 1.000 | 0.998,1.003 |
| β -carotene | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| N-3 | 1.064 | 0.995,1.139 | 1.041 | 0.944,1.149 |
| N-6 | 0.973 | 0.929,1.018 | 0.974 | 0.916,1.037 |
| n6:n3 | 0.856 | 0.721,1.017 | 0.915 | 0.719,1.165 |
| Steamed /grilled fish | 1.000 | 0.997,1.004 | 0.998 | 0.992,1.003 |
| Sea food | 1.010 | 0.965,1.058 | 0.983 | 0.913,1.060 |
| Pome fruits | 1.000 | 0.999,1.002 | 0.999 | 0.997,1.002 |
| Berry fruits | 0.993 | 0.979,1.007 | 0.984 | 0.963,1.007 |
| Citrus fruits | 1.000 | 0.996,1.003 | 0.995 | 0.989,1.001 |
| Stone fruits | 0.998 | 0.993,1.003 | 0.995 | 0.988,1.003 |
| Tropical fruits | 0.999 | 0.996,1.002 | 0.999 | 0.996,1.003 |
| Dried/preserved fruit | 0.994 | 0.978,1.010 | 0.997 | 0.975,1.014 |
| Fresh vegetables | 0.999 | 0.996,1.002 | 0.997 | 0.993,1.002 |

*Odds ratio adjusted for age, sex, BMI and total energy intake. ** Statistically significant (p<0.05).

3.1.4.3 Association between daily nutrient/antioxidant and food intake and atopy

The association between daily nutrient/antioxidant and food intake and atopy is described in Table 9. There were significant differences between mean dietary intakes of zinc and the ratio of n6:n3 between those who had atopy and those who didn't have atopy (Table 9).

Atopy positive adolescents had a significantly higher daily intake of zinc than atopy negative adolescents ($p=0.02$) (Table 9). Atopy positive adolescents also had a higher n6:n3 ratio than their atopy negative counterparts ($p=0.04$).

The unadjusted level of zinc was shown to have detrimental effects against atopy ($OR= 1.029$, 95% $CI= 1.004-1.055$). However after adjusting for confounders (age, sex, BMI and total energy intake) this association was proved to be insignificant ($OR= 1.019$, 95% $CI= 0.957-1.085$) (Table 10).

Increased intake of berry fruits showed protective effects against atopy (adjusted $OR= 0.988$, 95% $CI= 0.976-0.999$). Therefore, when we controlled for potential confounders, for every 1 g/day increased intake of these fruits there was a 1.2% reduction in the risk of having atopy.

Table 9: Daily nutrient/antioxidant intake for atopy positive and atopy negative, mean \pm SEM using independent samples T-test (n=1518).

| Food/Nutrient | Atopy Positive Mean (SEM) N=676 | Atopy Negative Mean (SEM) N=842 | <i>P-value</i> |
|--|--|--|-----------------------|
| *Vitamin A (μ g/day) | 3.06 (0.008) | 3.04 (0.007) | 0.21 |
| *Vitamin C (mg/day) | 2.19 (0.011) | 2.20 (0.009) | 0.81 |
| *Vitamin E (mg/day) | 0.89 (0.009) | 0.88 (0.008) | 0.93 |
| *Zinc (mg/day) | 1.09 (0.006) | 1.07 (0.005) | 0.02 |
| *Magnesium (mg/day) | 2.48 (0.006) | 2.47 (0.005) | 0.44 |
| * β -carotene (μ g/day) | 3.24 (0.025) | 3.25 (0.024) | 0.64 |
| *N-3 (g/day) | 0.07 (0.196) | 0.06 (0.167) | 0.23 |
| *N-6 (g/day) | 1.00 (0.009) | 1.01 (0.008) | 0.59 |
| *n6:n3 | 0.95 (0.006) | 0.93 (0.007) | 0.04 |
| Steamed /grilled fish (g/day) | 27.82 (1.90) | 25.35 (1.66) | 0.33 |
| Sea food (g/day) | 1.51 (0.15) | 1.44 (0.13) | 0.71 |
| *Pome fruits | 1.79 (0.02) | 1.80 (0.01) | 0.56 |
| Berry fruits | 7.02 (0.45) | 8.33 (0.59) | 0.09 |
| *Citrus fruits | 1.41 (0.022) | 1.40 (0.019) | 0.86 |
| *Stone fruits | 1.27 (0.025) | 1.29 (0.021) | 0.84 |
| *Tropical fruits | 1.57 (0.022) | 1.62 (0.017) | 0.098 |
| *Fruits combined (Pome, berry, citrus, stone) | 2.02 (0.019) | 2.03 (0.016) | 0.709 |
| *Mixture of 2 or more group of fruits | 1.25 (0.021) | 1.27 (0.018) | 0.457 |
| Dried/preserved fruit | 5.04 (0.61) | 4.52 (0.46) | 0.488 |
| *Fresh vegetables (g/day) | 1.86 (0.013) | 1.85 (0.012) | 0.754 |

(*logarithmic value of nutrients).

Table 10: Adjusted and unadjusted odds ratio (OR) for the risk of atopy for different foods/nutrients.

| Food/Nutrient | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|-----------------------|-----------------|--------------------|---------------------|--------------------|
| Vitamin A | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| Vitamin C | 1.000 | 0.999,1.001 | 0.999 | 0.998,1.001 |
| Vitamin E | 1.000 | 0.977,1.024 | 0.984 | 0.944,1.026 |
| Zinc | **1.029 | 1.004,1.055 | 1.019 | 0.957,1.085 |
| Magnesium | 1.000 | 0.999,1.001 | 1.001 | 0.999,1.003 |
| β-carotene | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| N-3 | 1.030 | 0.988,1.075 | 1.010 | 0.949,1.076 |
| N-6 | 0.978 | 0.949,1.007 | 1.000 | 0.958,1.043 |
| n6:n3 | 0.935 | 0.848,1.032 | 1.002 | 0.867,1.158 |
| Steamed /grilled fish | 1.001 | 0.999,1.003 | 1.001 | 0.998,1.004 |
| Sea food | 1.006 | 0.976,1.037 | 1.000 | 0.956,1.045 |
| Pome fruits | 1.000 | 0.999,1.001 | 0.999 | 0.997,1.000 |
| Berry fruits | 0.993 | 0.985,1.001 | **0.988 | 0.976,0.999 |
| Citrus fruits | 1.000 | 0.998,1.002 | 0.999 | 0.996,1.001 |
| Stone fruits | 1.000 | 0.997,1.003 | 0.999 | 0.994,1.003 |
| Tropical fruits | 1.000 | 0.998,1.002 | 0.999 | 0.997,1.001 |
| Dried/preserved fruit | 1.003 | 0.995,1.011 | 0.999 | 0.989,1.009 |
| Fresh vegetables | 1.000 | 0.998,1.002 | 0.999 | 0.996,1.001 |

*Odds ratio adjusted for age, sex, BMI and total energy intake. ** Statistically significant (p<0.05).

3.1.4.4 Association between daily nutrient/antioxidant and food intake and bronchial hyperresponsiveness.

The association between daily nutrient/antioxidant and food intake and bronchial hyperresponsiveness is described in Table 11. There were no significant differences between mean dietary intakes of vitamins A, E, C, magnesium, zinc β -carotene, n-3, n-6 fatty acids, steamed/grilled fish, sea foods and different fruits and vegetables between those who were BHR positive compared to those who were BHR negative.

The unadjusted level of n-3 fatty acid intake was showed protective effects against BHR (OR= 0.920, 95% CI= 0.868–0.974). However after adjusting for confounders (age, sex, BMI and total energy intake) this association was proved to be insignificant (OR= 0.933, 95% CI= 0.856–1.016) (Table 12).

Table 11: Daily nutrient/antioxidant intake for BHR positive and BHR negative, mean \pm SEM (n=1479)

| Food/Nutrient | BHR positive Mean (SEM) N=265 | BHR negative Mean (SEM) N=1214 | <i>P-value</i> |
|--|--|---|-----------------------|
| *Vitamin A (μ g/day) | 3.04 (0.013) | 3.05 (0.005) | 0.714 |
| Vitamin C (mg/day) | 191.78 (7.68) | 187.27 (3.43) | 0.585 |
| Vitamin E (mg/day) | 8.84 (0.315) | 8.77 (0.135) | 0.834 |
| Zinc (mg/day) | 12.53 (0.272) | 12.94 (0.134) | 0.200 |
| Magnesium (mg/day) | 307.46 (7.139) | 319.99 (3.469) | 0.128 |
| β -carotene (μ g/day) | 2419.11 (187.04) | 2626.89 (107.50) | 0.384 |
| N-3 (g/day) | 1.25 (0.036) | 1.30 (0.02) | 0.271 |
| N-6 (g/day) | 11.85 (0.457) | 11.71 (0.184) | 0.756 |
| n6:n3 | 9.70 (0.260) | 9.47 (0.106) | 0.429 |
| Steamed /grilled fish (g/day) | 24.68 (2.64) | 26.47 (1.42) | 0.591 |
| Sea food (g/day) | 1.20 (0.19) | 1.52 (0.11) | 0.143 |
| *Pome fruit (g/day) | 1.78 (0.03) | 1.79 (0.01) | 0.632 |
| Berry fruit (g/day) | 7.13 (0.738) | 8.19 (0.476) | 0.333 |
| Citrus fruit (g/day) | 34.26 (3.54) | 32.92 (1.69) | 0.740 |
| Stone fruit (g/day) | 22.32 (1.98) | 26.15 (1.12) | 0.093 |
| *Tropical fruit (g/day) | 1.61 (0.03) | 1.59 (0.01) | 0.581 |
| Fruits combined (Pome, berry, citrus, stone) (g/day) | 149.93 (9.07) | 150.82 (4.47) | 0.933 |
| *Mixture of 2 or more group of fruits (g/day) | 1.24 (0.03) | 1.27 (0.01) | 0.391 |
| Dried/preserved fruit (g/day) | 3.95 (0.76) | 4.99 (0.43) | 0.297 |
| Fresh vegetables (g/day) | 84.09 (3.47) | 87.81 (1.75) | 0.338 |

(*logarithmic value of nutrients).

Table 12: Adjusted and unadjusted odds ratio (OR) for the risk of BHR for different foods/nutrients.

| Food/Nutrient | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|-----------------------|-----------------|----------------------|---------------------|---------------|
| Vitamin A | 1.000 | 1.000,1.000 | 1.000 | 0.999,1.000 |
| Vitamin C | 1.000 | 0.999,1.002 | 1.001 | 0.999,1.002 |
| Vitamin E | 1.003 | 0.973,1.035 | 1.037 | 0.983,1.093 |
| Zinc | 0.979 | 0.947,1.012 | 0.931 | 0.857,1.012 |
| Magnesium | 1.000 | 0.999,1.001 | 1.001 | 0.998,1.003 |
| β-carotene | 1.000 | 1.000,1.000 | 1.000 | 1.000,1.000 |
| N-3 | 0.920 | **0.868,0.974 | 0.933 | 0.856,1.016 |
| N-6 | 0.999 | 0.961,1.039 | 0.973 | 0.922,1.028 |
| n6:n3 | 1.133 | 1.000,1.283 | 1.035 | 0.852,1.257 |
| Steamed /grilled fish | 0.999 | 0.96,1.002 | 0.998 | 0.994,1.003 |
| Sea food | 0.970 | 0.926,1.017 | 0.951 | 0.887,1.021 |
| Pome fruits | 1.000 | 0.999,1.002 | 1.001 | 0.999,1.003 |
| Berry fruits | 0.995 | 0.984,1.006 | 0.994 | 0.979,1.008 |
| Citrus fruits | 1.000 | 0.98,1.003 | 1.000 | 0.997,1.003 |
| Stone fruits | 0.997 | 0.992,1.001 | 0.995 | 0.989,1.001 |
| Tropical fruits | 1.000 | 0.998,1.002 | 1.000 | 0.998,1.003 |
| Dried/preserved fruit | 0.993 | 0.981,1.006 | 1.000 | 0.987,1.013 |
| Fresh vegetables | 0.999 | 0.996,1.001 | 1.001 | 0.997,1.004 |

*Odds ratio adjusted for age, sex, BMI and total energy intake. ** Statistically significant (p<0.05).

3.1.5 The effects of antioxidants on number of times wheezed

The effects of antioxidant intake on the number of times wheezed in the last 12 months were investigated in the study adolescents (Table 13). With the exception of berry fruits, there was no significant effect of fruit, vegetable and antioxidant intake on the number of times the child wheezed.

The mean intake of berry fruits for adolescents who had wheezed between 3 to 12 times within the last year was higher (10.08 g/day) compared to those who had wheezed more than 12 times in the last year (1.69 g/day) ($p=0.025$) (Table 13). This suggests that those adolescents who had wheezed the most in the last year had consumed 8.39 g/day less berry fruits compared with those who wheezed between 3 to 12 times within the last year.

The mean intake of berry fruits was higher (8.10 g/day) for adolescents who had not wheezed in the last 12 months compared to those who had wheezed more than 12 times in the last year ($p=0.053$). This result was approaching significance.

Table 13: Analysis of variance of different antioxidants for number of times wheezed over the last 12 months (n=1531).

| Number of times wheezed in the last 12 months N %(n) | Mean vitamin A intake µg /day (±SEM) | Mean vitamin C intake mg/day (±SEM) | Mean magnesium intake mg/day (±SEM) | Mean zinc intake mg/day (±SEM) | Mean of berry fruits intake g/day (±SEM) | Mean of citrus fruits intake g/day (±SEM) | Mean of pome fruits intake g/day (±SEM) | Mean of stone fruits intake g/day (±SEM) | Mean of tropical fruits intake g/day (±SEM) | Mean of combined fruits g/day (±SEM) | Mean of combined vegetables fruits g/day (±SEM) | Mean β – carotene intake µg/day (±SEM) |
|--|--|--|--|--|---|--|--|--|--|--|--|--|
| A None 1315 (85.9%) | 1232.78 (16.71) | 192.86 (3.10) | 319.63 (3.03) | 12.86 (0.12) | 8.10 (0.38) | 34.72 (1.51) | 84.01 (2.45) | 26.38 (1.06) | 59.11 (1.87) | 153.21 (3.96) | 88.18 (1.57) | 2556.85 (94.06) |
| B 1-2 129 (8.5%) | 1284.97 (64.19) | 186.40 (11.53) | 315.45 (10.04) | 12.85 (0.38) | 6.69 (1.81) | 30.46 (4.48) | 84.75 (7.75) | 22.41 (2.89) | 52.23 (5.09) | 144.31 (11.15) | 87.72 (4.11) | 2647.37 (307.91) |
| C 3-12 67 (4.4%) | 1344.16 (125.95) | 190.59 (13.85) | 331.78 (15.37) | 13.58 (0.62) | 10.08 (2.26) | 29.79 (6.06) | 83.41 (11.04) | 27.79 (4.31) | 58.39 (6.91) | 151.06 (17.63) | 91.02 (7.78) | 2438.04 (375.52) |
| D >12 20 (1.3%) | 1156.62 (143.05) | 169.20 (18.84) | 305.23 (19.05) | 12.76 (0.84) | 1.69 (0.66) | 48.47 (13.27) | 80.24 (15.25) | 23.07 (7.53) | 43.28 (9.01) | 153.47 (24.89) | 87.87 (12.45) | 3143.33 (931.45) |
| p-value | 0.387 (A,B) 0.164 (A,C) 0.599 (A,D) 0.542 (B,C) 0.407 (B,D) 0.252 (C,D) | 0.547 (A,B) 0.873 (A,C) 0.357 (A,D) 0.808 (B,C) 0.531 (B,D) 0.461 (C,D) | 0.689 (A,B) 0.380 (A,C) 0.565 (A,D) 0.331 (B,C) 0.703 (B,D) 0.348 (C,D) | 0.996 (A,B) 0.176 (A,C) 0.924 (A,D) 0.264 (B,C) 0.930 (B,D) 0.455 (C,D) | 0.305 (A,B) 0.280 (A,C) 0.053 (A,D) 0.127 (B,C) 0.158 (B,D) 0.025 (C,D) | 0.409 (A,B) 0.471 (A,C) 0.266 (A,D) 0.933 (B,C) 0.173 (B,D) 0.181 (C,D) | 0.930 (A,B) 0.956 (A,C) 0.850 (A,D) 0.921 (B,C) 0.833 (B,D) 0.889 (C,D) | 0.266 (A,B) 0.766 (A,C) 0.699 (A,D) 0.349 (B,C) 0.943 (B,D) 0.625 (C,D) | 0.272 (A,B) 0.931 (A,C) 0.291 (A,D) 0.541 (B,C) 0.576 (B,D) 0.372 (C,D) | 0.505 (A,B) 0.904 (A,C) 0.994 (A,D) 0.754 (B,C) 0.789 (B,D) 0.947 (C,D) | 0.931 (A,B) 0.687 (A,C) 0.980 (A,D) 0.700 (B,C) 0.991 (B,D) 0.827 (C,D) | 0.774 (A,B) 0.781 (A,C) 0.362 (A,D) 0.684 (B,C) 0.481 (B,D) 0.354 (C,D) |

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed

3.1.6 The relationship of RBC membrane fatty acids with the incidence of wheezing

The effects of RBC membrane fatty acids on number of times wheezed is shown in Table 14.

Analysing the effects of RBC fatty acids: linoleic acid, alpha-linolenic acid, parinaric acid, arachidonic acid, eicosapentaenoic acid, docosatetraenoic acid, docosapentaenoic acid, docosahexaenoic acid, total n-3, total n-6 and the ratio of n6:n3, on the number of times wheezed in the last 12 months showed that none of the n-6 fatty acids were associated with wheeze. However, significant associations between total n-3 fatty acids and wheezing were observed.

The mean level of α -linolenic acid (18:3n3) was equal for adolescents who had 1-2 episodes of wheezing and those who had 3-12 episodes within the last 12 months (0.21 mmol/L). This level was also very similar for with those with no wheeze (0.22 mmol/L). However, α -linolenic acid was approximately twice as much in those who had more than 12 wheezing episodes of wheezing in the last 12 months (0.41 mmol/L). Therefore, there were significant differences in the α -linolenic acid levels in the group with more than 12 wheezing episodes compared with the other 3 groups (Table 14).

The level of docosapentaenoic acid (22:5n3) was significantly higher for those adolescents with highest episodes of wheezing compared to those who did not wheeze in the last year ($p=0.035$)

The mean level of total n-3 fatty acids was overall significantly higher for those with the highest number of wheeze episodes compared to those with no wheeze (Table 14).

After performing regression analysis and adjusting for potential confounders (BMI, age, gender and total daily energy intake) none of the fatty acids were shown to be a predictor for wheezing (data not shown).

Table 14: Analysis of variance of the mean red blood cell membrane fatty acids for the number of times wheezed over the last 12 months (n=1531).

| Number of times wheezed in the last 12 months N %(n) | 18:3n3 Alpha-linolenic acid mmol/L (ALA) (±SEM) | 18:4n3 Parinaric acid mmol/L (±SEM) | 20:5n3 Eicosapentaenoic acid mmol/L (EPA) (±SEM) | 22:5n3 Docosapentaenoic acid mmol/L (DPA) (±SEM) | 22:6n3 Docosahexaenoic acid mmol/L (DHA) (±SEM) | 18:2n6 Linoleic acid mmol/L (±SEM) | 20:4n6 Arachidonic acid (AA) mmol/L (±SEM) | 22:4n6 Docosatetraenoic acid mmol/L (±SEM) | Total n-3 fatty acids mmol/L (±SEM) | Total n-6 fatty acids mmol/L (±SEM) | Ratio n6:n3 fatty acids (±SEM) |
|--|---|--|--|---|--|--|--|--|---|--|--|
| A None 1315 (85.9%) | 0.22 (0.007) | 0.53 (0.01) | 0.71 (0.006) | 4.29 (0.06) | 4.31 (0.03) | 10.08 (0.04) | 13.48 (0.06) | 1.59 (0.06) | 10.07 (0.08) | 30.03 (0.11) | 3.23 (0.03) |
| B 1-2 129 (8.5%) | 0.21 (0.19) | 0.54 (0.03) | 0.71 (0.019) | 4.61 (0.20) | 4.14 (0.09) | 10.12 (0.11) | 13.53 (0.21) | 1.49 (0.19) | 10.21 (0.22) | 30.00 (0.36) | 3.12 (0.09) |
| C 3-12 67 (4.4%) | 0.21 (0.02) | 0.53 (0.03) | 0.69 (0.02) | 4.77 (0.31) | 4.28 (0.15) | 9.91 (0.18) | 13.00 (0.27) | 1.84 (0.28) | 10.49 (0.39) | 29.45 (0.47) | 3.11 (0.15) |
| D >12 20 (1.3%) | 0.41 (0.007) | 0.63 (0.40) | 0.69 (0.04) | 5.37 (0.58) | 4.26 (0.25) | 9.63 (0.45) | 13.14 (0.57) | 1.05 (0.30) | 11.36 (0.73) | 28.57 (1.13) | 2.74 (0.25) |
| p-value | 0.545 (A,B) 0.778 (A,C) 0.002 (A,D) 0.879 (B,C) 0.001 (B,D) 0.003 (C,D) | 0.851 (A,B) 0.898 (A,C) 0.316 (A,D) 0.823 (B,C) 0.392 (B,D) 0.343 (C,D) | 0.807 (A,B) 0.470 (A,C) 0.666 (A,D) 0.663 (B,C) 0.762 (B,D) 0.982 (C,D) | 0.154 (A,B) 0.097 (A,C) 0.035 (A,D) 0.649 (B,C) 0.167 (B,D) 0.300 (C,D) | 0.127 (A,B) 0.853 (A,C) 0.834 (A,D) 0.416 (B,C) 0.416 (B,D) 0.926 (C,D) | 0.749 (A,B) 0.296 (A,C) 0.111 (A,D) 0.292 (B,C) 0.107 (B,D) 0.352 (C,D) | 0.812 (A,B) 0.099 (A,C) 0.506 (A,D) 0.135 (B,C) 0.475 (B,D) 0.817 (C,D) | 0.665 (A,B) 0.349 (A,C) 0.259 (A,D) 0.299 (B,C) 0.382 (B,D) 0.144 (C,D) | 0.617 (A,B) 0.230 (A,C) 0.036 (A,D) 0.506 (B,C) 0.080 (B,D) 0.209 (C,D) | 0.947 (A,B) 0.235 (A,C) 0.093 (A,D) 0.354 (B,C) 0.125 (B,D) 0.370 (C,D) | 0.319 (A,B) 0.415 (A,C) 0.060 (A,D) 0.971 (B,C) 0.178 (B,D) 0.208 (C,D) |

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed

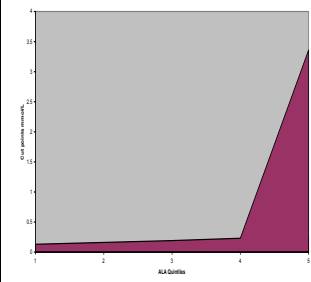
3.1.7 The effects of RBC fatty acids on asthmatic and atopic outcomes

A skin-prick test (SPT) was performed to determine the presence of atopic dermatitis in 1518 fourteen year old adolescents. The effects of different RBC fatty acids (linoleic, α -linolenic, parinaric, arachidonic, eicosapentaenoic acids and docosatetraenoic) were examined on asthmatic and atopic outcomes. These effects are described below and demonstrated in Tables 15-22. The graph within these Tables depicts the relationship between the cut points (mmol/L) and the quintiles for the corresponding fatty acid.

3.1.7.1 The effects of RBC α -linolenic acid (18:3n3) on asthmatic and atopic outcomes

The effects of RBC α -linolenic acid on asthmatic and atopic outcomes are described in Table 10. In the atopy positive group (n=584) there was a significant difference between each quintile as indicated by the Pearson chi ($p=0.043$) and there were more atopy positive adolescents in the fourth highest α -linolenic acid quintile. No associations were found between the other outcomes (current asthma, current wheeze, BHR) and the level of α -linolenic acid in RBC.

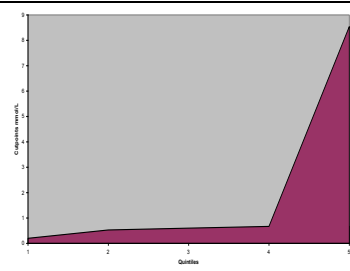
Table 15: Asthmatic and atopic outcome by quintile of alpha-linolenic acid (ALA) 18:3 n3 as measured in red blood cells.

| Alpha-linolenic acid (ALA) 18:3 n3 | | | | | | | | |
|------------------------------------|----------------|----------------|------------------|------------------|------------------|-----------------|---|---------------|
| Quintile | Total number | Lowest 1 | 2 | Middle 3 | 4 | Highest 5 |  | |
| Cut-points Range mmol/L | | 0.00 0.1300 | 0.1301 0.1600 | 0.1601 0.1900 | 0.1901 0.2300 | 0.2301 3.360 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 37 | 28 | 17 | 27 | 31 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 12.3 | 9.5 | 6.9 | 12.8 | 11.7 | 0.181 | 0.821 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 42 | 40 | 22 | 36 | 39 | | |
| Outcome within quintile (as %) | 13.6 | 14.0 | 13.5 | 8.9 | 17.1 | 14.7 | 0.135 | 0.529 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 130 | 137 | 94 | 111 | 112 | | |
| Outcome within quintile (as %) | 44.00 | 41.9 | 46.4 | 38.5 | 51.9 | 42.4 | 0.043 | 0.589 |
| Outcome | BHR | | | | | | | |
| n | 226 | 58 | 51 | 41 | 39 | 37 | | |
| Outcome within quintile (as %) | 17.8 | 19.9 | 18.1 | 17.6 | 19.0 | 14.2 | 0.488 | 0.137 |

3.1.7.2 The effects of RBC parinaric acid (18:4n3) on asthmatic and atopic outcomes

The effects of RBC parinaric acid on asthmatic and atopic outcomes are described in Table 16. In the atopy positive group (n=584) there was a significant difference between each quintile (p= 0.016) and there were more atopy positive adolescents in the third and fourth parinaric acid intake groups. There were no associations between current asthma, current wheeze, and BHR outcomes and the level of parinaric acid in RBC.

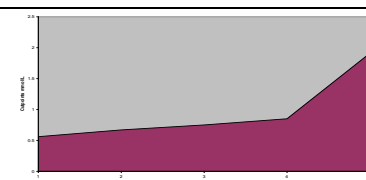
Table 16: Asthmatic and atopic outcome by quintile of parinaric acid 18:4 n3 as measured in red blood cells.

| Parinaric acid 18:4 n3 | | | | | | | | |
|--------------------------------|----------------|----------------|------------------|------------------|------------------|------------------|--|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.00 0.2000 | 0.2001 0.5300 | 0.5301 0.6000 | 0.6001 0.6700 | 0.6701 8.5400 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 25 | 26 | 27 | 33 | 29 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 9.9 | 8.8 | 10.3 | 13.0 | 11.3 | 0.568 | 0.229 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 32 | 35 | 34 | 40 | 38 | | |
| Outcome within quintile (as %) | 13.6 | 12.7 | 11.8 | 13.0 | 15.8 | 14.8 | 0.650 | 0.209 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 105 | 139 | 124 | 123 | 93 | | |
| Outcome within quintile (as %) | 44.0 | 40.4 | 47.0 | 48.1 | 48.0 | 36.2 | 0.016 | 0.437 |
| Outcome | BHR | | | | | | | |
| n | 226 | 40 | 49 | 40 | 52 | 45 | | |
| Outcome within quintile (as %) | 17.8 | 16.4 | 17.5 | 16.1 | 20.9 | 17.9 | 0.655 | 0.403 |

3.1.7.3 The effects of RBC eicosapentaenoic acid (20:5n3) on asthmatic and atopic outcomes

The effects of RBC eicosapentaenoic acid on asthmatic and atopic outcomes are described in Table 17. BHR was shown to be strongly associated with the level of eicosapentaenoic acid in RBC $p=0.007$. Eicosapentaenoic acid levels in RBC did not show any effects on current asthma, current wheeze and atopy.

Table 17: Asthmatic and atopic outcome by quintile of eicosapentaenoic acid 20:5 n3 as measured in red blood cells.

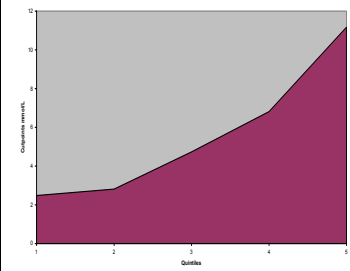
| Eicosapentaenoic acid 20:5 n3 | | | | | | | | |
|--------------------------------|----------------|--------------|--------------|--------------|--------------|--------------|---|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.00 0.56 | 0.57 0.67 | 0.68 0.75 | 0.76 0.85 | 0.86 1.91 | | |
| | | | | | | | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 33 | 32 | 21 | 32 | 22 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 12.00 | 11.9 | 8.2 | 12.1 | 8.7 | 0.388 | 0.289 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 38 | 43 | 29 | 41 | 28 | | |
| Outcome within quintile (as %) | 13.6 | 13.8 | 15.9 | 11.3 | 15.5 | 11.1 | 0.336 | 0.384 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 126 | 121 | 112 | 117 | 108 | | |
| Outcome within quintile (as %) | 44.0 | 44.8 | 45.0 | 44.1 | 44.3 | 41.7 | 0.945 | 0.474 |
| Outcome | BHR | | | | | | | |
| n | 226 | 55 | 29 | 38 | 50 | 54 | | |
| Outcome within quintile (as %) | 17.8 | 22.2 | 11.1 | 15.5 | 19.5 | 20.6 | 0.007 | 0.141 |

3.1.7.4 The effects of RBC docosapentaenoic acid (22:5n3) on asthmatic and atopic outcomes

The effects of RBC docosapentaenoic acid on asthmatic and atopic outcomes are shown in Table 18. There were significant differences between current asthma, current wheeze and BHR and the lowest and the highest of the quintiles of red blood cell levels of docosapentaenoic acid. For adolescents with current asthma, the lowest quintile of docosapentaenoic acid had 8.2% of the sample population while the highest quintile consisted of 12.7% of the group ($p=0.106$, trend p -value 0.013) (Table 18). For current wheeze positive cases, the lowest quintile of docosapentaenoic acid had 10.9% while the highest quintile consisted of 17.2% of the cases ($p=0.166$, trend p -value 0.014).

Within the bronchial hyperresponsiveness (BHR) positive cases 19.2% were in the lowest quintile of RBC docosapentaenoic acid levels, while the highest quintile included only 15.2% of the cases ($p=0.90$, trend p -value 0.029).

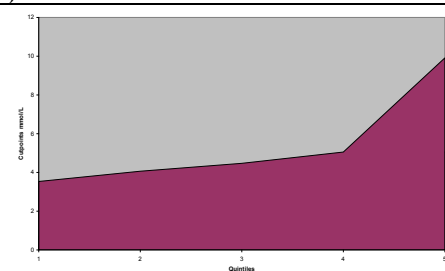
Table 18: Asthmatic and atopic outcome by quintile of docosapentaenoic acid (DPA) 22:5 n3 as measured in red blood cells.

| Docosapentaenoic acid (DPA) 22:5 n3 | | | | | | | | |
|-------------------------------------|----------------|--------------|--------------|--------------|--------------|---------------|---|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.29 2.48 | 2.49 2.81 | 2.82 4.73 | 4.74 6.81 | 6.82 11.17 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 21 | 20 | 29 | 36 | 34 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 8.2 | 7.5 | 11.1 | 13.3 | 12.7 | 0.106 | 0.013 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 28 | 29 | 36 | 40 | 46 | | |
| Outcome within quintile (as %) | 13.6 | 10.9 | 10.9 | 13.8 | 14.8 | 17.2 | 0.166 | 0.014 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 113 | 112 | 109 | 128 | 122 | | |
| Outcome within quintile (as %) | 44.0 | 43.5 | 42.3 | 40.8 | 47.4 | 46.0 | 0.530 | 0.282 |
| Outcome | BHR | | | | | | | |
| n | 226 | 46 | 59 | 44 | 38 | 39 | | |
| Outcome within quintile (as %) | 17.8 | 19.2 | 22.8 | 17.5 | 17.4 | 15.2 | 0.900 | 0.029 |

3.1.7.5 The effects of RBC docosahexaenoic acid (22:6n3) on asthmatic and atopic outcomes

The effects of RBC docosahexaenoic acid on asthmatic and atopic outcomes are shown in Table 19. RBC docosahexaenoic acid level showed significant differences between BHR positive and the lowest and the highest of the quintiles. For adolescents who were BHR positive, the lowest quintile of RBC docosahexaenoic acid level contained 20.9% of the group, while the highest quintile consisted of only 14.8% of the BHR positive group (p=0.242, trend p-value 0.036).

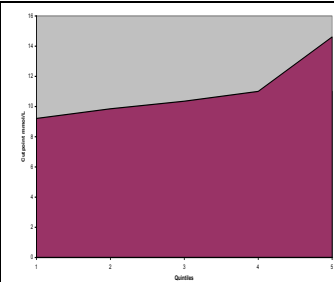
Table 19: Asthmatic and atopic outcome by quintile of docosahexaenoic acid (DHA) 22:6 n3 as measured in red blood cells.

| Docosahexaenoic acid (DHA) 22:6 n3 | | | | | | | | |
|------------------------------------|----------------|--------------|--------------|--------------|--------------|--------------|---|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.69 3.53 | 3.54 4.06 | 4.07 4.47 | 4.48 5.05 | 5.06 9.91 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 30 | 30 | 26 | 27 | 27 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 11.3 | 11.5 | 9.8 | 10.3 | 10.2 | 0.963 | 0.584 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 37 | 39 | 39 | 34 | 30 | | |
| Outcome within quintile (as %) | 13.6 | 20.7 | 21.8 | 21.8 | 19.0 | 16.8 | 0.756 | 0.291 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 123 | 115 | 98 | 119 | 129 | | |
| Outcome within quintile (as %) | 44 | 45.6 | 43.4 | 37.3 | 45.4 | 48.3 | 0.117 | 0.437 |
| Outcome | BHR | | | | | | | |
| n | 226 | 55 | 51 | 40 | 42 | 38 | | |
| Outcome within quintile (as %) | 17.8 | 20.9 | 20.5 | 15.7 | 16.9 | 14.8 | 0.242 | 0.036 |

3.1.7.6 The effects of RBC linoleic acid on asthmatic (18:2n6) and atopic outcomes

The effects of RBC linoleic acid in quintiles, on asthmatic and atopic outcomes are shown in Table 20. The RBC level of linoleic acid was divided into quintiles and the proportion of the sample which showed the outcome of interest was recorded for each quintile. No significant trend was found between the lowest and the highest quintiles of linoleic acid levels and any of the asthmatic and allergic outcomes. Interestingly if the lowest quintile was ignored there was a weak trend in the increasing of the proportion of each quintile with increasing the level of linoleic acid for atopy (Table 15).

Table 20: Asthmatic and atopic outcome by quintile of linoleic acid (LA) 18:2 n6 as measured in red blood cells.

| Linoleic acid (LA) 18:2 n6 | | | | | | | | |
|--------------------------------|----------------|----------------|------------------|-------------------|--------------------|--------------------|--|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.00 9.2100 | 9.2101 9.8500 | 9.8501 10.3500 | 10.3501 11.0020 | 11.0021 14.6100 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 32 | 26 | 27 | 25 | 30 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 12.0 | 9.7 | 10.7 | 9.5 | 11.2 | 0.872 | 0.774 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 44 | 34 | 32 | 32 | 37 | | |
| Outcome within quintile (as %) | 13.6 | 16.5 | 12.7 | 12.6 | 12.1 | 13.9 | 0.595 | 0.382 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 117 | 103 | 118 | 122 | 124 | | |
| Outcome within quintile (as %) | 44.0 | 42.4 | 38.9 | 45.9 | 46.4 | 46.6 | 0.303 | 0.098 |
| Outcome | BHR | | | | | | | |
| n | 226 | 48 | 40 | 39 | 44 | 55 | | |
| Outcome within quintile (as %) | 17.8 | 18.1 | 15.8 | 15.9 | 17.2 | 21.7 | 0.399 | 0.263 |

3.1.7.7 The effects of RBC arachidonic acid (20:4n6) on asthmatic and atopic outcomes

The effects of RBC arachidonic acid on asthmatic and atopic outcomes are described in Table 21. There was no evidence to suggest any associations between the level of arachidonic acid in RBC and any of the outcomes of interest.

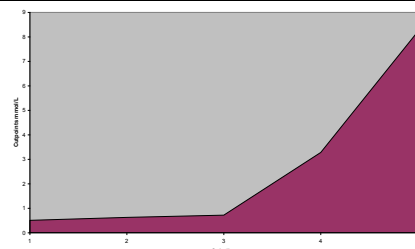
Table 21: Asthmatic and atopic outcome by quintile of arachidonic acid (AA) 20:4n6 as measured in red blood cells.

| Arachidonic acid (AA) 20:4n6 | | | | | | | |
|--|-----------------------|-------------|------|-------------|-------|--------------|--------------------|
| Quintile | Total number | Lowest 1 | 2 | Middle 3 | 4 | Highest 5 | |
| Cut-points | | 0.00 | 0.57 | 0.68 | 0.76 | 0.86 | |
| Range mmol/L | | 0.56 | 0.67 | 0.75 | 0.85 | 1.91 | |
|  | | | | | | | |
| Outcome | Current asthma | | | | | | |
| n | 140 | 36 | 25 | 30 | 24 | 25 | Pearson chi square |
| Outcome within quintile (as %) | 10.6 | 13.6 | 9.5 | 11.5 | 9.1 | 9.4 | Trend p-value |
| | | | | | | | 0.383 |
| | | | | | | | 0.138 |
| Outcome | Current wheeze | | | | | | |
| n | 179 | 44 | 34 | 33 | 34 | 34 | |
| Outcome within quintile (as %) | 13.6 | 16.7 | 12.9 | 12.6 | 12.9 | 12.7 | |
| | | | | | | | 0.608 |
| | | | | | | | 0.241 |
| Outcome | Atopy | | | | | | |
| n | 584 | 127 | 118 | 117 | 111 | 111 | |
| Outcome within quintile (as %) | 44.0 | 47.4 | 44.4 | 45.0 | 41.3 | 42.0 | |
| | | | | | | | 0.628 |
| | | | | | | | 0.151 |
| Outcome | BHR | | | | | | |
| n | 226 | 48 | 52 | 43 | 34 | 49 | |
| Outcome within quintile (as %) | 17.8 | 18.3 | 20.2 | 17.3 | 13.41 | 19.6 | |
| | | | | | | | 0.295 |
| | | | | | | | 0.575 |

3.1.7.8 The effects of RBC docosatetraenoic acid (22:4n6) on asthmatic and atopic outcomes

The effects of RBC docosatetraenoic acid on asthmatic and atopic outcomes are shown in Table 22. There was no evidence to suggest any associations between the level of docosatetraenoic acid in RBC and any of the outcomes of interest.

Table 22: Asthmatic and atopic outcome by quintile of docosatetraenoic acid 22:4 n6 as measured in red blood cells.

| Docosatetraenoic acid 22:4 n6 | | | | | | | | |
|--------------------------------|----------------|--------------|--------------|--------------|--------------|--------------|--|---------------|
| | | Lowest | | Middle | | Highest |  | |
| Quintile | Total number | 1 | 2 | 3 | 4 | 5 | | |
| Cut-points Range mmol/L | | 0.00 0.51 | 0.52 0.63 | 0.64 0.72 | 0.73 3.28 | 3.29 8.21 | | |
| Outcome | Current asthma | | | | | | | |
| n | 140 | 26 | 32 | 35 | 22 | 25 | Pearson chi square | Trend p-value |
| Outcome within quintile (as %) | 10.6 | 9.8 | 11.2 | 13.7 | 8.6 | 9.8 | 0.373 | 0.662 |
| Outcome | Current wheeze | | | | | | | |
| n | 179 | 33 | 42 | 42 | 29 | 33 | | |
| Outcome within quintile (as %) | 13.6 | 12.4 | 14.7 | 16.5 | 11.3 | 12.9 | 0.446 | 0.712 |
| Outcome | Atopy | | | | | | | |
| n | 584 | 113 | 133 | 121 | 120 | 97 | | |
| Outcome within quintile (as %) | 44.0 | 42.8 | 46.5 | 47.1 | 46.9 | 36.7 | 0.079 | 0.214 |
| Outcome | BHR | | | | | | | |
| n | 226 | 52 | 45 | 47 | 34 | 48 | | |
| Outcome within quintile (as %) | 17.8 | 20.2 | 16.4 | 19.3 | 13.5 | 19.5 | 0.253 | 0.568 |

3.1.8 Comparison of RBC membrane fatty acid content in wheezers and non-wheezers

The level of different fatty acids in RBC for wheezers versus non-wheezers was compared in adolescents (Table 23). It was found that the concentration of docosatetraenoic acid (22:4n6) was significantly lower for wheezers (4.987 mmol/L) compared with non-wheezers (5.056 mmol/L) ($p=0.047$). No other fatty acids, total n-3 fatty acids, total n-6 fatty acids and the ratio of n6:n3 showed significant effects on wheezing.

The unadjusted level of docosapentaenoic acid (22:5n3) was shown to have detrimental effects against wheezing (OR= 1.096, 95% CI= 1.020–1.178). However after adjusting for confounders (age, sex, BMI and total energy intake) this association was proved to be insignificant (OR= 1.077, 95% CI= 0.966–1.200) (Table 24).

The adjusted ratio of n6:n3 was shown to have detrimental association on wheezing episodes (OR= 1.064, 95% CI= 1.007–1.124). Therefore, while controlling for potential confounders, for every 1 unit increased in the ratio of n6:n3 there was a 6.4% increase in the risk of having wheezing episodes.

Table 23: Red blood cell Fatty acids levels for wheezers and non-wheezers mean (SEM) using T-tests.

| Red blood cell fatty acids | Wheezers (Mean \pmSEM) (mmol/L) | Non-wheezers (Mean \pmSEM) (mmol/L) | <i>P-value</i> |
|---------------------------------------|---|---|-----------------------|
| 18:2n6 Linoleic acid | 9.928 (0.806) | 9.965 (0.032) | 0.661 |
| 18:3n3 Alpha-linolenic acid (ALA) | 0.224 (0.019) | 0.206 (0.005) | 0.137 |
| 18:4n3 Parinaric acid | 0.618 (0.015) | 0.617 (0.006) | 0.507 |
| 20:4n6 Arachidonic acid (AA) | 13.274 (0.142) | 13.313 (0.062) | 0.911 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.697 (0.013) | 0.696 (0.006) | 0.248 |
| 22:4n6 Docosatetraenoic acid | 4.987 (0.083) | 5.056 (0.030) | 0.047 |
| 22:5n3 Docosapentaenoic acid (DPA) | 2.370 (0.031) | 2.389 (0.013) | 0.439 |
| 22:6n3 Docosahexaenoic acid (DHA) | 4.143 (0.071) | 4.206(0.029) | 0.759 |
| n-3 | 8.053 (0.093) | 8.113 (0.038) | 0.993 |
| n-6 | 32.923 (0.186) | 33.084 (0.081) | 0.772 |
| n6:n3 | 4.147 (0.059) | 4.150 (0.025) | 0.738 |

Table 24: Odds ratio (OR) for the risk of current wheeze in red blood cell Fatty acids

| Red blood cell fatty acids | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|---------------------------------------|-----------------|--------------------|---------------------|--------------------|
| 18:2n6 Linoleic acid | 0.947 | 0.832,1.076 | 1.006 | 0.836,1.211 |
| 18:3n3 Alpha-linolenic acid (ALA) | 1.118 | 0.617,2.024 | 1.377 | 0.607,3.121 |
| 18:4n3 Parinaric acid | 1.047 | 0.719,1.524 | 0.852 | 0.387,1.877 |
| 20:4n6 Arachidonic acid (AA) | 0.968 | 0.902,1.038 | 0.990 | 0.895,1.095 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.768 | 0.359,1.639 | 0.695 | 0.226,2.134 |
| 22:4n6 Docosatetraenoic acid | 0.993 | 0.917,1.075 | 0.980 | 0.870,1.105 |
| 22:5n3 Docosapentaenoic acid (DPA) | **1.096 | 1.020,1.178 | 1.077 | 0.966,1.200 |
| 22:6n3 Docosahexaenoic acid (DHA) | 0.910 | 0.784,1.055 | 0.908 | 0.738,1.116 |
| n-3 | 1.092 | 0.874,1.364 | 0.987 | 0.672,1.451 |
| n-6 | 1.018 | 0.994,1.041 | 1.030 | 0.992,1.069 |
| n6:n3 | 1.036 | 0.994,1.080 | **1.064 | 1.007,1.124 |

*Data are presented as odds ratio (OR) (95% confidence interval). OR are adjusted for the following confounders: Age, sex, BMI and total energy intake. **Statistically significant (p< 0.05).

3.1.9 Comparison of RBC membrane fatty acid content in asthmatic and non-asthmatic adolescents

The mean level of RBC fatty acid was compared between asthmatic and non-asthmatic adolescents (Table 25). No significant differences in the concentration of fatty acids between the asthmatic and non-asthmatic adolescents were found.

The unadjusted level of docosapentaenoic acid (22:5n3) was shown to have detrimental effects on asthma (OR= 1.112, 95% CI= 1.027–1.204) (Table 25). However after adjusting for confounders (age, sex, BMI and total energy intake) this association was proved to be insignificant (OR= 1.103, 95% CI= 0.980–1.241) (Table 26).

The adjusted ratio of n6:n3 was shown to have a detrimental association with asthma (OR= 1.072, 95% CI= 1.009–1.139). Therefore, while controlling for potential confounders, for every 1 level increased in the ratio of n6:n3 there was a 7.2% increase in risk of developing asthma.

Table 25: Red blood cell fatty acids levels for asthma and non-asthma cases mean (SEM) using T-tests.

| Red blood cell fatty acid | Current asthma (Mean \pm SEM) (mmol/L) | Non-asthma (Mean \pm SEM) (mmol/L) | <i>P-value</i> |
|---------------------------------------|--|--|-----------------------|
| 18:3n3 Alpha-linolenic acid (ALA) | 0.216 (0.025) | 0.199 (0.069) | 0.446 |
| 18:4n3 Parinaric acid | 0.601 (0.017) | 0.622 (0.009) | 0.669 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.721 (0.023) | 0.689 (0.007) | 0.904 |
| 22:5n3 Docosapentaenoic acid (DPA) | 2.333 (0.042) | 2.385 (0.016) | 0.190 |
| 22:6n3 Docosahexaenoic acid (DHA) | 4.178 (0.112) | 4.218 (0.038) | 0.926 |
| 18:2n6 Linoleic acid | 9.857 (0.128) | 9.970 (0.040) | 0.326 |
| 20:4n6 Arachidonic acid (AA) | 13.575 (0.246) | 13.289 (0.085) | 0.735 |
| 22:4n6 Docosatetraenoic acid | 4.975 (0.143) | 5.045 (0.042) | 0.128 |
| n-3 | 8.049 (0.137) | 8.114 (0.050) | 0.829 |
| n-6 | 33.151 (0.295) | 33.012 (0.113) | 0.639 |
| n6:n3 | 4.189 (0.065) | 4.145 (0.024) | 0.651 |

Table 26: Odds ratio (OR) for the risk of current asthma in red blood cell Fatty acids

| Red blood cell fatty acids | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|---------------------------------------|-----------------|--------------------|---------------------|--------------------|
| 18:2n6 Linoleic acid | 0.976 | 0.845,1.128 | 1.018 | 0.831,1.246 |
| 18:3n3 Alpha-linolenic acid (ALA) | 1.197 | 0.637,2.248 | 1.393 | 0.583,3.329 |
| 18:4n3 Parinaric acid | 1.019 | 0.63,1.566 | 0.862 | 0.363,2.044 |
| 20:4n6 Arachidonic acid (AA) | 0.961 | 0.890,1.038 | 0.950 | 0.855,1.054 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.645 | 0.276,1.508 | 0.501 | 0.147,1.710 |
| 22:4n6 Docosatetraenoic acid | 0.987 | 0.902,1.078 | 0.987 | 0.868,1.123 |
| 22:5n3 Docosapentaenoic acid (DPA) | **1.112 | 1.027,1.204 | 1.103 | 0.980,1.241 |
| 22:6n3 Docosahexaenoic acid (DHA) | 0.930 | 0.789,1.096 | 0.883 | 0.706,1.105 |
| n-3 | 1.062 | 0.825,1.367 | 1.076 | 0.707,1.638 |
| n-6 | 1.009 | 0.983,1.036 | 1.033 | 0.992,1.077 |
| n6:n3 | 1.030 | 0.983,1.079 | **1.072 | 1.009,1.139 |

*Data are presented as odds ratio (OR) (95% confidence interval). OR are adjusted for the following confounders: Age, sex, BMI and total energy intake. **Statistically significant ($p < 0.05$).

3.2 Comparison of RBC membrane fatty acid content in atopy and non-atopy adolescents

The mean level of RBC fatty acid was compared between atopic and non-atopic adolescents (Table 27). The mean level of α -linolenic acid (18:3n3) was higher ($p=0.016$) and docosapentaenoic acid (22:5n3) was lower ($p=0.039$) in red blood cell membrane in atopic compared with non-atopic adolescents. None of the other differences between atopic and non-atopic cases were shown to be significant.

The unadjusted level of docosatetraenoic acid (22:4n6) was shown to be protective against atopy (OR= 0.946, 95% CI= 0.895–0.999) (Table 27). However after adjusting for confounders (age, sex, BMI and total energy intake) this protection was proved to be insignificant (OR= 0.968, 95% CI= 0.892–1.050) (Table 28).

Table 27: Red blood cell fatty acids levels for atopy and non-atopy cases mean (SEM) using T-tests.

| Red blood cell fatty acids | Atopy Mean (SEM) (mmol/L) | Non-atopy Mean (SEM) (mmol/L) | <i>P-value</i> |
|---------------------------------------|----------------------------------|--------------------------------------|-----------------------|
| 18:3n3 Alpha-linolenic acid (ALA) | 0.219 (0.013) | 0.190 (0.006) | 0.016 |
| 18:4n3 Parinaric acid | 0.619 (0.013) | 0.621 (0.012) | 0.733 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.699 (0.012) | 0.694 (0.009) | 0.946 |
| 22:5n3 Docosapentaenoic acid (DPA) | 2.374 (0.027) | 2.377 (0.019) | 0.039 |
| 22:6n3 Docosahexaenoic acid (DHA) | 4.247 (0.059) | 4.185 (0.046) | 0.155 |
| 18:2n6 Linoleic acid | 9.932 (0.061) | 9.993 (0.051) | 0.798 |
| 20:4n6 Arachidonic acid (AA) | 13.328 (0.129) | 13.297 (0.105) | 0.874 |
| 22:4n6 Docosatetraenoic acid | 5.042 (0.065) | 5.013 (0.054) | 0.459 |
| n-3 | 8.158 (0.077) | 8.067 (0.059) | 0.159 |
| n-6 | 32.990 (0.163) | 33.010 (0.139) | 0.779 |
| n6:n3 | 4.129 (0.037) | 4.162 (0.029) | 0.251 |

Table 28: Odds ratio (OR) for the risk of atopy in red blood cell Fatty acids

| Red blood cell fatty acids | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|---------------------------------------|-----------------|--------------------|---------------------|---------------|
| 18:2n6 Linoleic acid | 1.057 | 0.966,1.157 | 1.068 | 0.938,1.216 |
| 18:3n3 Alpha-linolenic acid (ALA) | 0.965 | 0.623,1.495 | 0.907 | 0.463,1.777 |
| 18:4n3 Parinaric acid | 0.818 | 0.620,1.080 | 0.906 | 0.532,1.544 |
| 20:4n6 Arachidonic acid (AA) | 0.980 | 0.932,1.029 | 1.012 | 0.942,1.088 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.835 | 0.500,1.394 | 1.056 | 0.486,2.296 |
| 22:4n6 Docosatetraenoic acid | **0.946 | 0.895,0.999 | 0.968 | 0.892,1.050 |
| 22:5n3 Docosapentaenoic acid (DPA) | 1.041 | 0.990,1.095 | 0.985 | 0.913,1.063 |
| 22:6n3 Docosahexaenoic acid (DHA) | 1.055 | 0.954,1.166 | 1.120 | 0.972,1.291 |
| n-3 | 1.085 | 0.922,1.277 | 1.134 | 0.862,1.491 |
| n-6 | 0.995 | 0.978,1.012 | 0.987 | 0.959,1.016 |
| n6:n3 | 0.975 | 0.976,1.004 | 0.979 | 0.940,1.019 |

*Data are presented as odds ratio (OR) (95% confidence interval). OR are adjusted for the following confounders: Age, sex, BMI and total energy intake. **Statistically significant (p < 0.05).

3.2.1 Comparison of red blood cell fatty acid content in BHR positive and BHR negative adolescents.

The mean level of RBC fatty acids was compared between BHR positive and BHR negative adolescents (Table 29). The only n-6 fatty acid which had a higher level in BHR positive compared with BHR negative adolescents was docosatetraenoic acid (22:4n6). The other n-6 fatty acids (linoleic 18:2n6 and arachidonic 20:4n6) and total n-6 level were lower in BHR positive compared with BHR negative individuals. The level of the majority of the individual n-3 fatty acids (parinaric 18:4n3, eicosapentaenoic 20:5n3 and docosapentaenoic 22:5n3) and total n-3 fatty acids was higher in BHR positive compared with BHR negative cases except for α -linolenic acid (18:3n3) and docosahexaenoic acid (22:6n3) which had lower levels in BHR positive adolescents although none of these differences reached significance.

The unadjusted level of docosapentaenoic acid (22:5n3) was shown to be protective against BHR (OR= 0.928, 95% CI= 0.866–0.995). However after adjusting for confounders (age, sex, BMI and total energy intake) this association was not significant (OR= 0.965, 95% CI= 0.871–1.070) (Table 30).

Increased level of docosahexaenoic acid (22:6n3) in RBC membrane showed protective effects against BHR (adjusted OR= 0.814, 95% CI= 0.669–0.989). Therefore, when controlled for potential confounders, for every 1 mmol/L increased level of docosahexaenoic acid in RBC membrane there was an 18.6% reduction in risk of having BHR.

Table 29: Red blood cell fatty acids levels for BHR positive and negative cases mean (SEM) using T-tests.

| Red blood cell fatty acids | BHR+ Mean (SEM) (mmol/L) | BHR- Mean (SEM) (mmol/L) | <i>P-value</i> |
|---------------------------------------|---|---|-----------------------|
| 18:3n3 Alpha-linolenic acid (ALA) | 0.186 (0.008) | 0.209 (0.008) | 0.070 |
| 18:4n3 Parinaric acid | 0.637 (0.022) | 0.617 (0.098) | 0.710 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 0.701 (0.022) | 0.695 (0.078) | 0.248 |
| 22:5n3 Docosapentaenoic acid (DPA) | 2.403 (0.035) | 2.379 (0.017) | 0.629 |
| 22:6n3 Docosahexaenoic acid (DHA) | 4.209 (0.089) | 4.213 (0.039) | 0.525 |
| 18:2n6 Linoleic acid | 9.832 (0.090) | 10.001 (0.043) | 0.874 |
| 20:4n6 Arachidonic acid (AA) | 13.043 (0.192) | 13.405 (0.086) | 0.949 |
| 22:4n6 Docosatetraenoic acid | 5.140 (0.096) | 5.002 (0.047) | 0.525 |
| n-3 | 8.135 (0.018) | 8.114 (0.051) | 0.513 |
| n-6 | 32.656 (0.253) | 33.134 (0.116) | 0.816 |
| n6:n3 | 4.098 (0.058) | 4.153 (0.024) | 0.952 |

Table 30: Odds ratio (OR) for the risk of BHR in red blood cell Fatty acids

| Red blood cell fatty acids | Crude OR | 95% CI | *Adjusted OR | 95% CI |
|---------------------------------------|-----------------|--------------------|---------------------|--------------------|
| 18:2n6 Linoleic acid | 1.038 | 0.921,1.171 | 1.001 | 0.842,1.190 |
| 18:3n3 Alpha-linolenic acid (ALA) | 0.455 | 0.193,1.071 | 0.529 | 0.155,1.809 |
| 18:4n3 Parinaric acid | 0.756 | 0.486,1.177 | 1.182 | 0.597,2.341 |
| 20:4n6 Arachidonic acid (AA) | 1.017 | 0.948,1.092 | 0.973 | 0.886,1.070 |
| 20:5n3 Eicosapentaenoic acid (EPA) | 1.243 | 0.632,2.444 | 0.648 | 0.228,1.839 |
| 22:4n6 Docosatetraenoic acid | 1.017 | 0.948,1.092 | 0.929 | 0.822,1.050 |
| 22:5n3 Docosapentaenoic acid (DPA) | **0.928 | 0.866,0.995 | 0.965 | 0.871,1.070 |
| 22:6n3 Docosahexaenoic acid (DHA) | 0.883 | 0.771,1.012 | **0.814 | 0.669,0.989 |
| n-3 | 0.879 | 0.698,1.106 | 0.885 | 0.601,1.303 |
| n-6 | 1.004 | 0.981,1.026 | 1.024 | 0.988,1.062 |
| n6:n3 | 1.017 | 0.978,1.058 | 1.027 | 0.975,1.082 |

*Data are presented as odds ratio (OR) (95% confidence interval). OR are adjusted for the following confounders: Age, sex, BMI and total energy intake. **Statistically significant (p < 0.05).

3.2.2 Asthma severity; antioxidants and fatty acids intake

The asthma severity outcomes (described in section 2.5.3) were divided into four groups: severe asthma (2.5%), moderate asthma (4.4%), mild asthma (4.0%) and no asthma (89.1%). The effects of different antioxidants and fatty acids on the severity of asthma were investigated.

3.2.2.1 The effects of dietary antioxidant, fruits and vegetables and severity of asthma

The individual intake of 13 fresh vegetables (carrots, turnip, broad beans, green beans, capsicum, cabbages, green peas, Brussels sprouts, spinach, broccoli, cauliflower, pumpkins, sweet corn, and zucchini) did not show a significant association with asthma severity in adolescents. However, when the intake of the combination of these vegetables was examined against asthma severity a significant non protective association was shown (Table 31). The mean intake of combined vegetables in individuals with severe asthma was higher at 102.45 g/day compared with that for moderate asthma (73.64 g/day). The difference between the mean intake of these combined vegetables was 28.81g/day higher ($p=0.044$) for severe asthma ($n=38$) compared with moderate asthma ($n=67$) (Figure 8). The mean intake of combined vegetables for mild asthma was 84.96 g/day and for moderate asthma was 73.64 g/day. Therefore, the difference between the mean intake of these combined vegetables was 11.32 g/day higher for mild asthma ($n=62$) compared with moderate asthma ($n=67$), although this difference was not statistically significant ($p= 0.359$).

Table 31: Analysis of variance of the effect of dietary antioxidant, fruits and vegetables on severity of asthma (n=1531).

| Asthma severity | Mean vitamin A intake µg /day (±SEM) | Mean vitamin C intake mg/day (±SEM) | Mean vitamin E intake mg/day (±SEM) | Mean magnesium intake mg/day (±SEM) | Mean zinc intake mg/day (±SEM) | Mean of berry fruits intake g/day (±SEM) | Mean of citrus fruits intake g/day (±SEM) | Mean of pome fruits intake g/day (±SEM) | Mean of stone fruits intake g/day (±SEM) | Mean of tropical fruits intake g/day (±SEM) | Mean of combined fruits g/day (±SEM) | *Mean β – carotene intake µg/day (±SEM) | Mean of combined vegetables fruits g/day (±SEM) |
|--|--|--|--|--|--|--|--|--|---|--|--|--|---|
| A No asthma 1364 (89.1%) | 1235.34 (17.97) | 190.54 (3.26) | 8.74 (0.13) | 318.16 (3.20) | 12.84 (0.12) | 8.07 (0.42) | 32.85 (1.55) | 84.17 (2.64) | 25.86 (1.03) | 58.19 (1.97) | 160.77 (5.40) | 2629.68 (79.62) | 88.79 (1.97) |
| B Mild asthma 62 (4.0%) | 1251.98 (84.96) | 179.56 (17.74) | 9.20 (0.65) | 300.70 (14.66) | 12.61 (0.59) | 5.02 (1.12) | 36.80 (10.16) | 96.54 (11.11) | 22.35 (4.46) | 51.61 (8.69) | 175.24 (24.87) | 2599.41 (333.38) | 84.96 (8.85) |
| C Moderate asthma 67 (4.4%) | 1111.58 (62.31) | 180.62 (14.99) | 9.31 (0.69) | 318.62 (15.71) | 12.93 (0.63) | 7.72 (1.54) | 25.29 (4.22) | 79.75 (12.49) | 22.98 (4.09) | 56.54 (7.78) | 130.32 (14.28) | 2000.6 (278.89) | 73.64 (5.42) |
| D Severe asthma 38 (2.5%) | 1285.79 (264.60) | 17.80 (15.01) | 8.86 (0.76) | 325.6 (20.45) | 13.41 (0.85) | 7.98 (3.84) | 36.29 (6.71) | 85.46 (14.40) | 25.85 (6.67) | 47.65 (7.93) | 152.38 (23.19) | 2035.97 (366.84) | 102.45 (16.19) |
| None, Mild None, Mod None, Severe Mild, Mod Mild, Severe Mod, Severe p-value | 0.859 (A,B) 0.161 (A,C) 0.002 (A,D) 0.265(B,C) 0.022 (B,D) 0.001 (C,D) | 0.498 (A,B) 0.516 (A,C) 0.520 (A,D) 0.961 (B,C) 0.944 (B,D) 0.908 (C,D) | 0.469 (A,B) 0.345 (A,C) 0.881 (A,D) 0.902 (B,C) 0.728 (B,D) 0.641 (C,D) | 0.273 (A,B) 0.976 (A,C) 0.700 (A,D) 0.402 (B,C) 0.313 (B,D) 0.770 (C,D) | 0.704 (A,B) 0.885 (A,C) 0.452 (A,D) 0.700 (B,C) 0.403 (B,D) 0.606 (C,D) | 0.146 (A,B) 0.860(A,C) 0.971(A,D) 0.337 (B,C) 0.364(B,D) 0.936(C,D) | 0.605 (A,B) 0.295 (A,C) 0.713 (A,D) 0.263 (B,C) 0.966 (B,D) 0.343 (C,D) | 0.343 (A,B) 0.719 (A,C) 0.936 (A,D) 0.338 (B,C) 0.584 (B,D) 0.773 (C,D) | 0.489(A,B) 0.546 (A,C) 0.998 (A,D) 0.927 (B,C) 0.657 (B,D) 0.709 (C,D) | 0.495(A,B) 0.856 (A,C) 0.372 (A,D) 0.704 (B,C) 0.792 (B,D) 0.544(C,D) | 0.562 (A,B) 0.189 (A,C) 0.791 (A,D) 0.176 (B,C) 0.564 (B,D) 0.567 (C,D) | 0.934 (A,B) 0.083 (A,C) 0.219 (A,D) 0.235 (B,C) 0.344 (B,D) 0.953 (C,D) | 0.680 (A,B) 0.078 (A,C) 0.246(A,D) 0.359 (B,C) 0.235(B,D) 0.044 (C,D) |

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed.

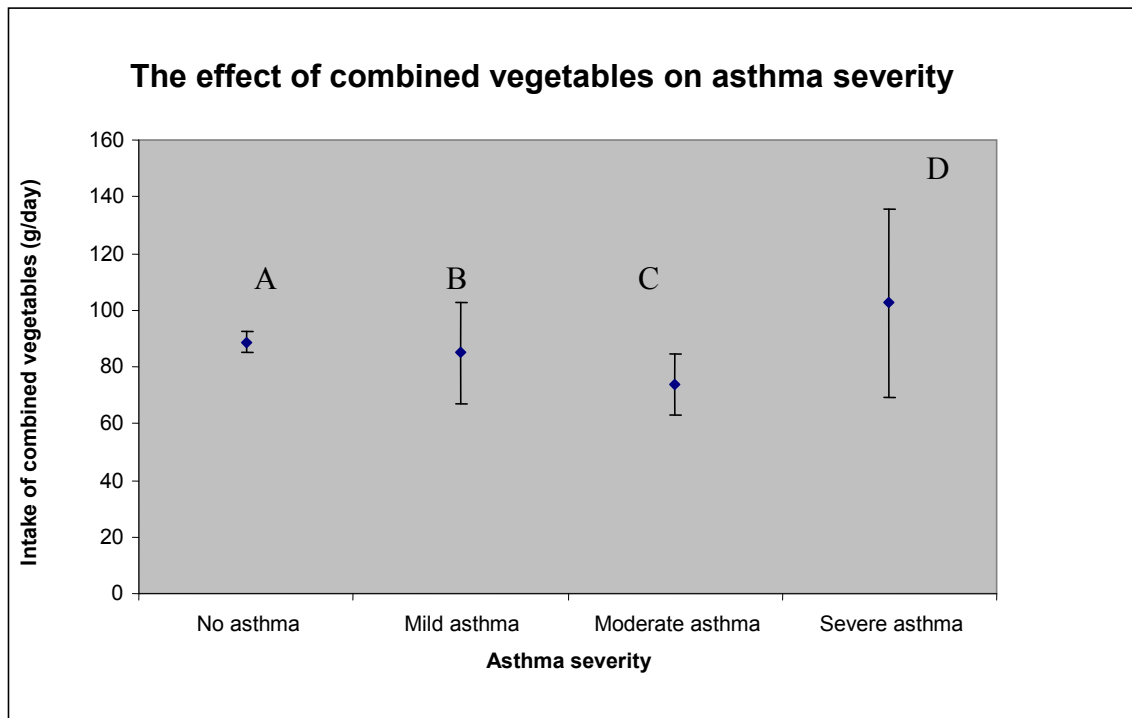


Figure 9 - The mean intake of combined vegetables (+/- 95% confidence intervals) and asthma severity at 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between mild and moderate asthma ($p=0.044$).

A: No asthma, B: Mild asthma, C: Moderate asthma, D: Severe asthma

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.680 |
| A, C | 0.078 |
| A, D | 0.246 |
| B, C | 0.359 |
| B, D | 0.235 |
| C, D | 0.044 |

There was a significant difference ($p=0.044$) in combined vegetables intake between the severe asthma group ($n=38$) compared with moderate asthma group ($n=67$) (Figure 9). The severe asthma group had a higher mean intake of combined vegetables compared with moderate asthma group. In Figures 8 to 11, despite observing overlaps between the Confidence Interval (CI) bars, significant differences were observed in the intake of foods and nutrients in groups with varying levels of asthma severity. This is because the Standard Error (SE) of a difference between two estimates is not simply the sum of the individual SE but a smaller value given by the square root of the sum of the squares of the individual values. Therefore, if 95% CI bars do not overlap, we can be sure that the difference is statistically significant

($P < 0.05$). However, the converse is not true, i.e. we may or may not have statistical significance when the 95% CI overlaps. This is different for SE bars, as when SE bars overlap, we can be sure the difference between the two means is not statistically significant ($P > 0.05$).

There was a significant difference ($p=0.001$) in vitamin A intake between the severe asthma group compared with no asthma, mild asthma and moderate asthma groups (Figure 10). The severe asthma group ($n=38$) had a higher mean intake of vitamin A compared with all the other groups. This difference in intake of vitamin A was especially high between severe and moderate asthma ($n=67$) with a mean difference of 474.21 $\mu\text{g/day}$.

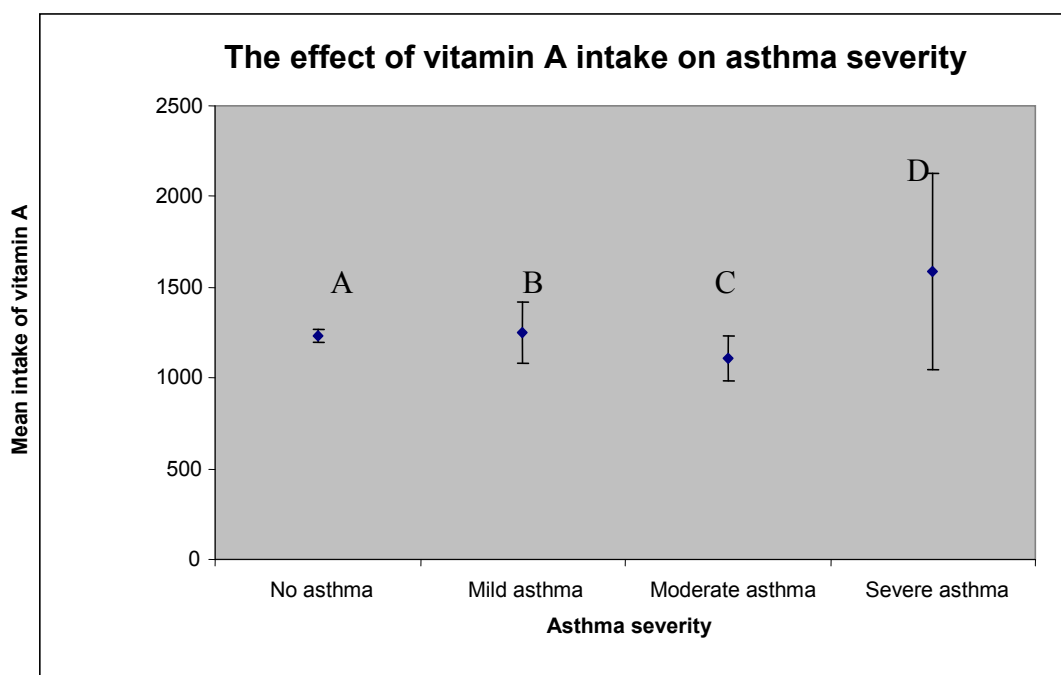


Figure 10 - The mean intake of vitamin A (\pm 95% confidence intervals) and severity of asthma at 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between no asthma and severe asthma ($p=0.002$), mild and severe asthma ($p=0.022$) and moderate and severe asthma ($p=0.001$).

A: No asthma, B: Mild asthma, C: Moderate asthma, D: Severe asthma

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.859 |
| A, C | 0.161 |
| A, D | 0.002 |
| B, C | 0.265 |
| B, D | 0.022 |
| C, D | 0.001 |

There was also a significant difference in vitamin A consumption between severe asthma and the no asthma group (n=1364) as the severe asthma group had a 350.45 µg/day (p=0.002) higher mean intake of vitamin A compared with the no asthma group (Figure 10). The difference in intake of vitamin A in the severe asthma compared with the mild asthma group (n=62) was not as high or as significant as in the other groups. The severe asthma group had a 333.81 µg/day (p=0.022) higher mean intake of vitamin A compared to the moderate asthma group.

3.2.2.2 The effects of fish consumption and fatty acid intake on asthma severity

The effect of fish consumption and asthma severity is shown in Figure 10.

Consumption of fish had a significant association with the severity of asthma. The average consumption of steamed/grilled fish intake for the group with severe asthma was 40.58 g/day; while for the group with moderate asthma was 20.50 g/day. Therefore, the severe asthma group (n=33) had 20.08 g/day, (p=0.039) higher mean intake of steamed/grilled fish intake compared with the group with moderate asthma (n=58) (Figure 11).

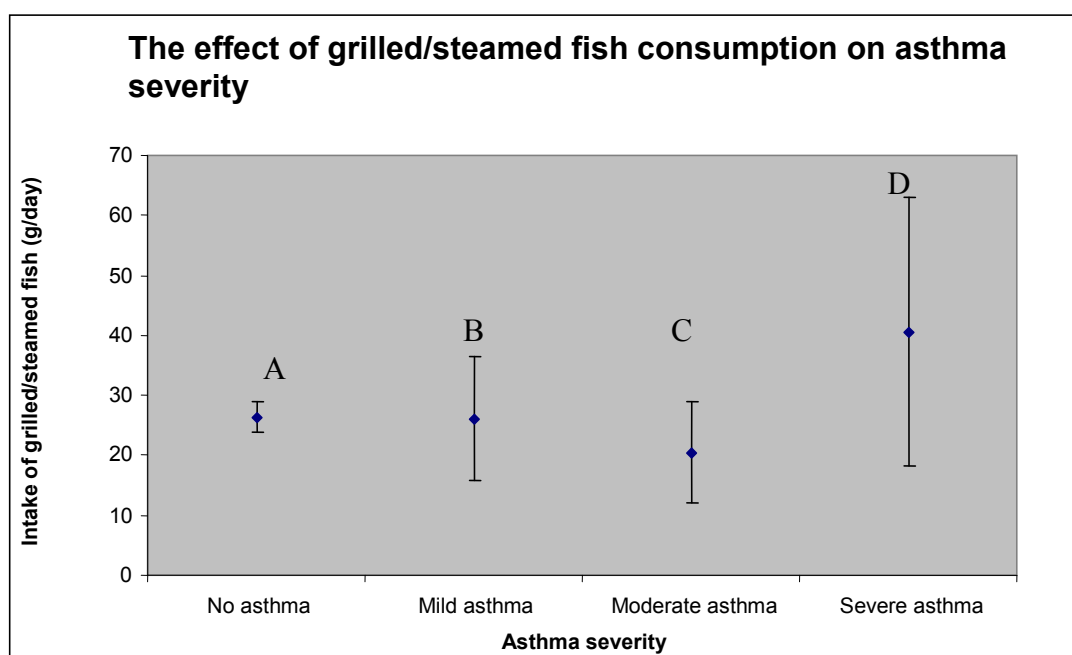


Figure 11 - The mean intake of grilled/steamed fish intake (+/- 95% confidence intervals) and severity of asthma at 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between moderate and severe asthma ($p=0.039$).

A: No asthma, B: Mild asthma, C: Moderate asthma, D: Severe asthma.

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.976 |
| A, C | 0.338 |
| A, D | 0.068 |
| B, C | 0.516 |
| B, D | 0.146 |
| C, D | 0.039 |

Red blood cell docosapentaenoic acid (22:5n3) level for the no asthma group (4.31 mmol/L) was significantly lower compared with the severe asthma group (5.14 mmol/L) ($p=0.027$) (Table 32).

Whilst the α -linolenic acid (18:3n3) in the no asthma group (0.22 mmol/L) was lower than in the severe asthma group (0.29 mmol/L), this difference was not statistically significant ($p=0.089$). In contrast the arachidonic acid (20:4n6) levels in the no asthma (13.62 mmol/L) group were higher than in the moderate asthma group (12.94 mmol/L) although this difference was also not statistically significant ($p=0.077$).

Table 32: Analysis of variance of different mean red blood cell membrane fatty acids (mmol/L) with severity of asthma (n=1319).

| Asthma severity N % (n) | 18:3n3 Alpha-linolenic acid (ALA) (±SEM) | 18:4n3 Parinaric acid (±SEM) | 20:5n3 Eicosapent aenoic acid (EPA) (±SEM) | 22:5n3 Docosapent aenoic acid (DPA) (±SEM) | 22:6n3 Docosahexa enoic acid (DHA) (±SEM) | 18:2n6 Linoleic acid (±SEM) | 20:4n6 Arachidonic acid (AA) (±SEM) | 22:4n6 Docosatetra enoic acid (±SEM) | Steam/grill ed fish (g/day) (±SEM) | *Total n-3 (±SEM) | *Total n-6 (±SEM) | *Ratio n6:n3 (±SEM) |
|---|--|---|--|---|--|--|--|--|---|--|--|--|
| A No asthma 1175 (89.1%) | 0.22 (0.007) | 0.54 (0.01) | 0.71 (0.006) | 4.31 (0.06) | 4.30 (0.03) | 10.08 (0.03) | 13.48 (0.06) | 1.59 (0.05) | 26.29 (1.30) | 10.08 (0.07) | 30.03 (0.11) | 3.23 (0.03) |
| B Mild asthma 53 (4.0%) | 0.23 (0.03) | 0.55 (0.04) | 0.71 (0.02) | 4.73 (0.28) | 4.25 (0.12) | 10.13 (0.16) | 13.62 (0.27) | 1.60 (0.27) | 26.10 (5.18) | 10.47 (0.30) | 30.21 (0.46) | 3.06 (0.14) |
| C Moderate asthma 58 (4.4%) | 0.19 (0.02) | 0.52 (0.03) | 0.68 (0.03) | 4.69 (0.31) | 4.24 (0.17) | 9.99 (0.17) | 12.94 (0.30) | 1.65 (0.28) | 20.50 (4.17) | 10.32 (0.38) | 29.29 (0.50) | 3.11 (0.15) |
| D Severe asthma 33 (2.5%) | 0.29 (0.45) | 0.57 (0.05) | 0.69 (0.02) | 5.14 (0.38) | 4.14 (0.19) | 9.98 (0.29) | 13.30 (0.40) | 1.27 (0.28) | 40.58 (11.05) | 10.83 (0.49) | 29.33 (0.75) | 2.95 (0.19) |
| None, Mild None, Mod None, Severe Mild, Mod Mild, Severe Mod, Severe p-value | 0.713 (A,B) 0.443 (A,C) 0.089 (A,D) 0.414 (B,C) 0.270 (B,D) 0.066 (C,D) | 0.876 (A,B) 0.729 (A,C) 0.668 (A,D) 0.717 (B,C) 0.812 (B,D) 0.575(C,D) | 0.952 (A,B) 0.255 (A,C) 0.539 (A,D) 0.440 (B,C) 0.656 (B,D) 0.814 (C,D) | 0.167 (A,B) 0.205 (A,C) 0.027 (A,D) 0.920 (B,C) 0.390 (B,D) 0.339 (C,D) | 0.731 (A,B) 0.673 (A,C) 0.386 (A,D) 0.959 (B,C) 0.644 (B,D) 0.339 (C,D) | 0.755 (A,B) 0.633 (A,C) 0.639 (A,D) 0.569 (B,C) 0.569 (B,D) 0.945 (C,D) | 0.651 (A,B) 0.077 (A,C) 0.643 (A,D) 0.110 (B,C) 0.511 (B,D) 0.450 (C,D) | 0.983 (A,B) 0.834 (A,C) 0.356 (A,D) 0.893 (B,C) 0.458 (B,D) 0.386 (C,D) | 0.976 (A,B) 0.338 (A,C) 0.068 (A,D) 0.516 (B,C) 0.146 (B,D) 0.039 (C,D) | 0.287 (A,B) 0.510 (A,C) 0.096 (A,D) 0.761 (B,C) 0.528 (B,D) 0.365 (C,D) | 0.727 (A,B) 0.143 (A,C) 0.275 (A,D) 0.192 (B,C) 0.277 (B,D) 0.950 (C,D) | 0.293 (A,B) 0.436 (A,C) 0.150 (A,D) 0.834 (B,C) 0.646 (B,D) 0.517 (C,D) |

Fisher's LSD p-value was used to compare between two groups means after ANOVA was performed.

The mean red blood cell membrane content of docosapentaenoic acid (DPA) (22:5n3) was 0.83 mmol/L higher in group with severe asthma (n=33) compared with the group with no asthma (n=1175) (p=0.027) (Figure 12)

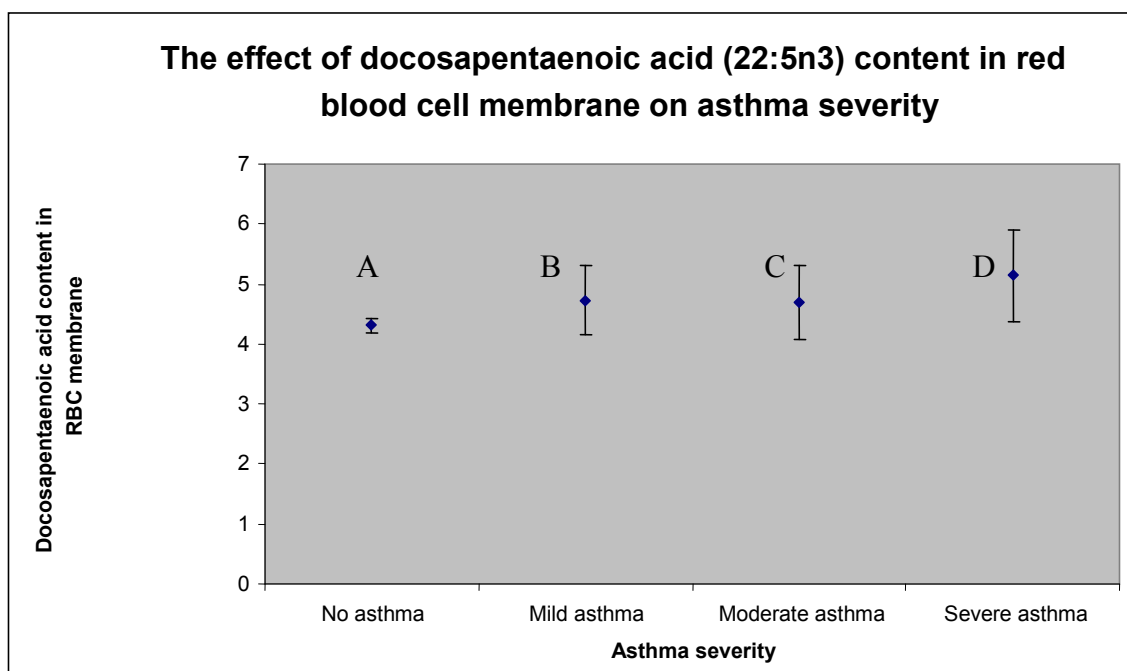


Figure 12- The mean level of red blood cell docosapentaenoic acid (22:5:n3) (+/- 95% confidence intervals) and asthma severity at 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant difference between groups A, D (p=0.027)

A: No asthma, B: Mild asthma, C: Moderate asthma, D: Severe asthma.

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.167 |
| A, C | 0.205 |
| A, D | 0.027 |
| B, C | 0.920 |
| B, D | 0.390 |
| C, D | 0.339 |

3.2.3 The effects of dietary antioxidant, fruits and vegetables on wheeze.

The effects of dietary antioxidant, fruits and vegetables on wheeze was summarised earlier in this chapter (section 3.1.4.1). Among the entire antioxidant nutrient and fruit intake, only the mean level of berry fruits intake showed significant associations with wheezing episodes within the last 12 months.

The mean intake of vitamin C was not normally distributed, therefore logarithmic values of the mean intake of vitamin C (which had a very close to normal distribution) were used for analysis in association with the number of times the adolescent had wheezed in the last 12 months (Figures 13 & 14). Comparison of mean intake of vitamin C for different groups based on the number of times wheezed in last 12 months demonstrated that there was no difference in vitamin C intake in adolescents and the number of times wheezed in the last 12 months (group A, B, C, D).

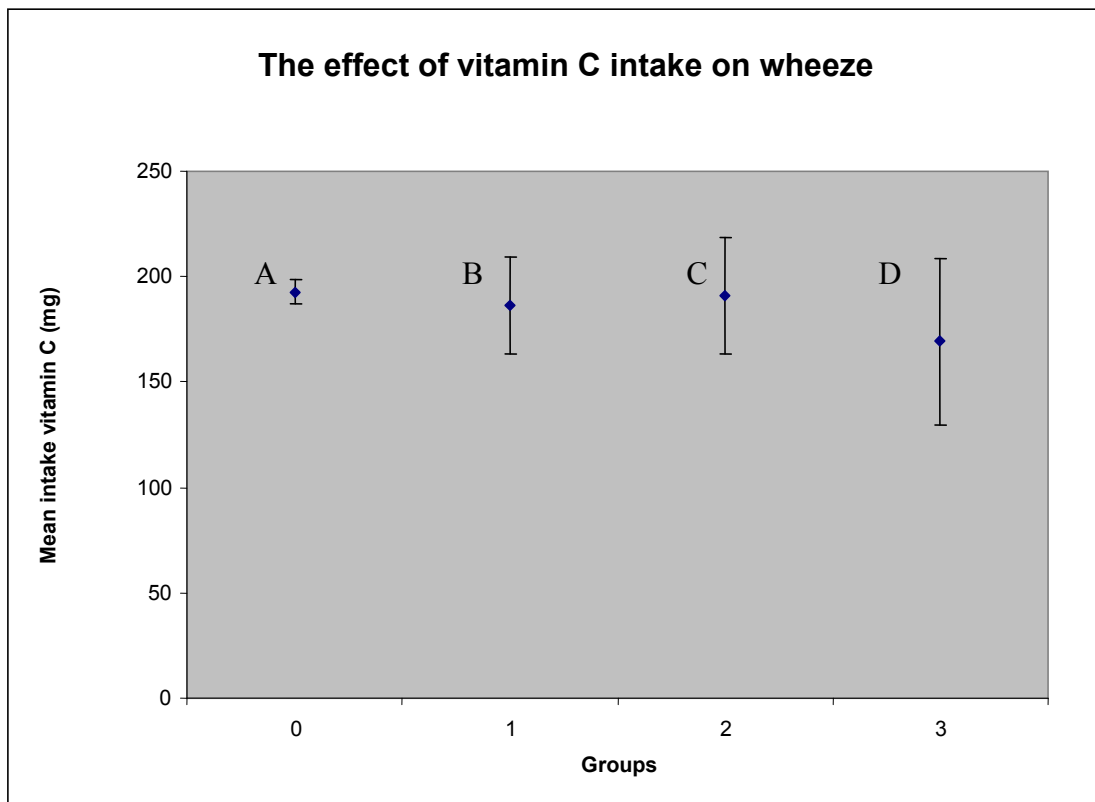


Figure 13 - The mean intake of vitamin C (+/- 95% confidence intervals) at 14 years of age and the number of times wheezed in the last 12 months.

The number of times wheezed in the last 12 months for each corresponding group: A; none, B; 1-2, C: 3-12 and D >12. Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed.

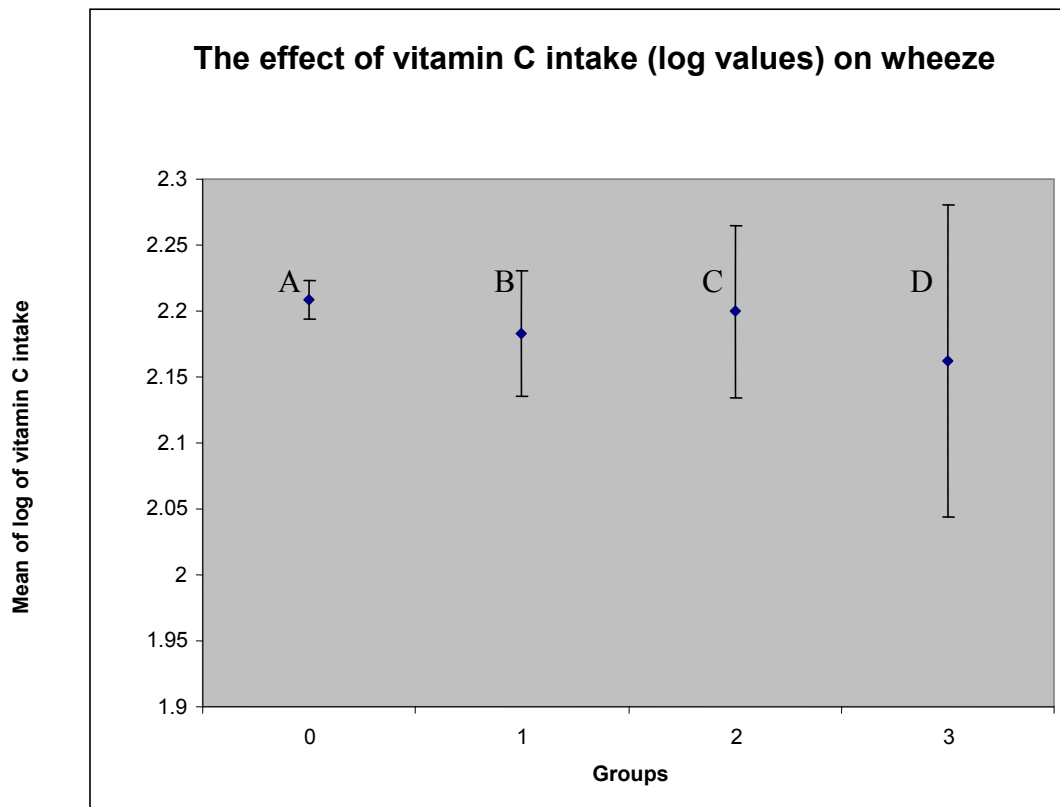


Figure 14 - The log of mean intake of vitamin intake C (+/- 95% confidence intervals) at 14 years of age and the number of times wheezed in the last 12 months.

The number of times wheezed in the last 12 months for each corresponding group: A; none, B; 1-2, C: 3-12 and D >12. Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed.

The mean intake of β -carotene was not normally distributed; therefore logarithmic values of the mean intake of β -carotene (which had a very close to normal distribution) were used for analysis in association with the number of times the adolescent had wheezed in the last 12 months (Figures 15 & 16). There was no difference in β -carotene intake in adolescents and the number of times wheezed in the last 12 months (group A, B, C, D).

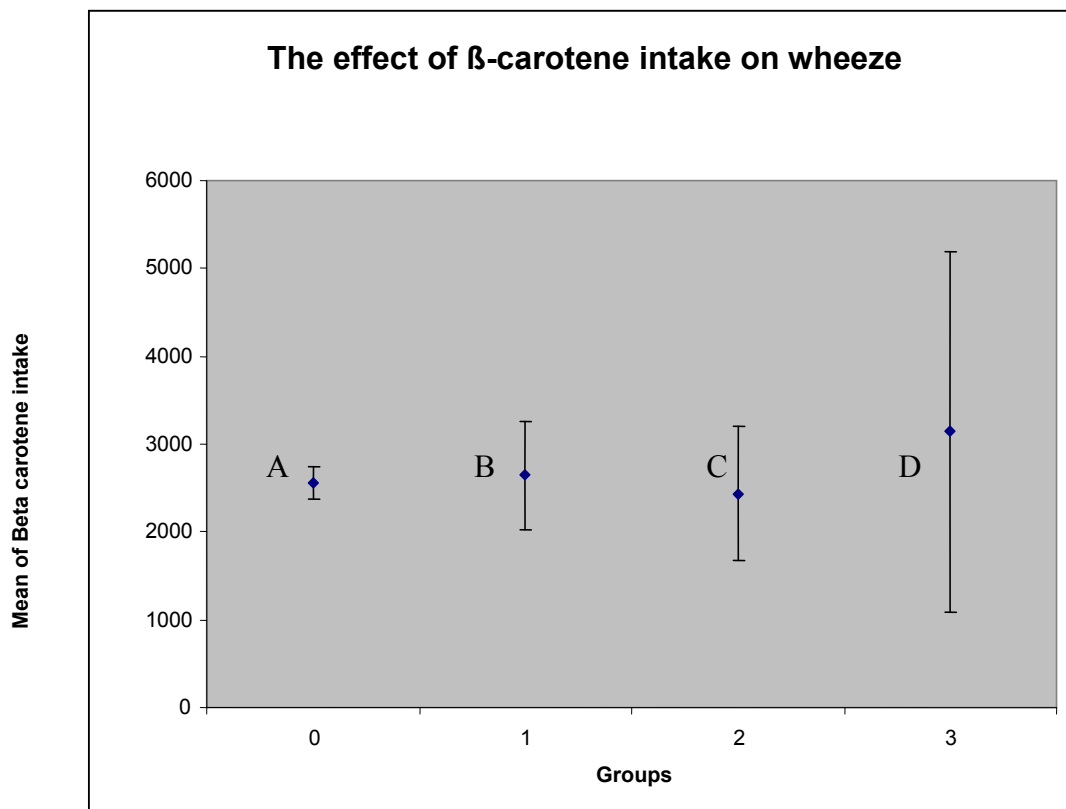


Figure 15 - The mean intake of β -carotene ($\mu\text{g/day}$) (+/- 95% confidence intervals) at 14 years of age and the number of times wheezed in the last 12 months.

The number of times wheezed in the last 12 months for each corresponding group: A; none, B; 1-2, C: 3-12 and D >12. Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed.

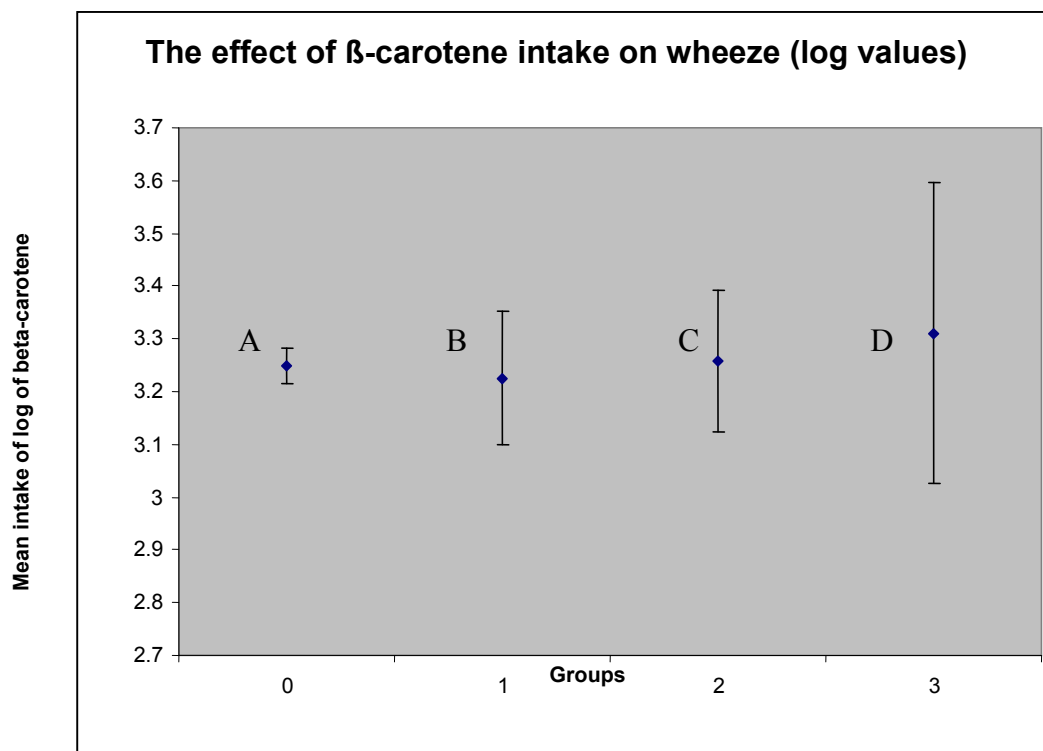


Figure 16 - The mean intake of log of β -carotene ($\mu\text{g/day}$) (\pm 95% confidence intervals) at 14 years of age and the number of times wheezed in the last 12 months.

The number of times wheezed in the last 12 months for each corresponding group: A: none, B: 1-2, C: 3-12 and D: >12. Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed.

3.3 THE EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS INTAKE ON ASTHMATIC SYMPTOMS (FFQ DATA)-STUDY ONE

In the first part of Study One using the data collected in FFQ, the effects of antioxidants and fatty acids intake on asthmatic symptoms were investigated. One of the main conclusions that can be drawn from the first part of this study is that most of the dietary antioxidants did not show a protective effect on asthma in 14 year old adolescents. One of the significant effects was observed in zinc consumption with average zinc intake higher for the atopy positive (1.09 mg/day) compared with the atopy negative group (1.07 mg/day) ($p=0.02$) (Table 9) suggesting that a higher zinc intake may be related to atopy. However, after controlling for confounders (age, sex, BMI and total energy intake) this effect was shown as non-significant (Table 10). This finding contradicts the proposed role of zinc as an antioxidant by inhibiting

oxidative stress-responsive events in endothelial cells (Hennig et al. 1999; Zalewski et al. 2005).

Daily consumption of berry fruits was negatively correlated with asthma severity based on wheezing frequency. The average daily intake of berry fruits for adolescents who had between 3-12 wheezing episodes within the last 12 months was significantly higher (10.08 g/day) than for those who had more than 12 wheezing episodes (1.69 g/day) (Table 13). The adjusted odds ratio for the risk of atopy also showed a protective role for berry fruits consumption (adjusted OR= 0.988, 95% CI= 0.976–0.999) (Table 10). Therefore, for every 1 g/day increased intake of berry fruits there was a 1.2% reduction in the risk of developing atopy ($1 - 0.988 \text{ (OR)} = 0.012 \times 100 = 1.2\%$).

Asthma severity (defined as mild, moderate, or severe) was another asthmatic symptom that was investigated in relation to adolescents' diet. The group with severe asthma had the highest vitamin A intake which was significantly higher compared to those with no asthma and also in comparison with those who had mild and moderate asthma (Table 31). Therefore, our crude analysis suggested that vitamin A does not protect against severity of asthma. After controlling for confounders we did not find any associations between the level of vitamin A daily intake and asthmatic symptoms. Hence, our findings on Vitamin A consumption and its effect on asthma severity contradicts the hypothesis that vitamin A, as a part of the antioxidant system, has a protective role against asthma (de Luis et al. 2005; Hatch 1995; McKeever & Britton 2004; Soutar, Seaton & Brown 1997). There are however other studies that associate high serum levels of vitamin A with increased risk of IgE sensitization (Kull et al. 2006) suggesting the need for further work in this area. In the absence of certain types of childhood infection, vitamin A and other retinoids accumulate in the lung. Later on, after exposure to known triggers for asthma, retinoid metabolites may be produced in high concentration and they produce an acute, localized form of retinoid intoxication, recognized as status asthmaticus (Mawson 2001). The underlying mechanism for this is yet to be known. In one study it has been demonstrated that in young adult mice excessive supplementary amounts of vitamin A (above dietary requirements) would enhance inflammatory responses accompanied by decreased T_H1 and increased mucosal responses (Albers et al. 2003). However,

supplementation of the same amount of vitamin A if combined with vitamins C and E, selenium, or zinc has no effects on immune function (Albers et al. 2003).

In analysing the role of different antioxidants on bronchial hyperresponsiveness (BHR) outcome, no significant differences were observed between BHR positive and BHR negative and the amount of intake of these nutrients. Our finding on the role of antioxidants on BHR supports other studies in which no association of manganese and vitamin C on BHR was observed (Soutar, Seaton & Brown 1997). However, we observed a mild protective association between the consumption of vitamin C on current asthma (adjusted OR= 0.997, 95% CI= 0.994–0.999). This means that when controlled for potential confounders, for every 1 mg/day increased intake of vitamin C there was a 0.3% reduction in the risk of developing asthma ($1 - 0.997$ (OR)= $0.003 \times 100 = 0.3\%$) (Table 8).

One of the major findings in relation to dietary fatty acid intake in Study One was observed in the ratio of n6:n3 fatty acids between the atopy positive and the atopy negative group. The ratio of n6:n3 fatty acids intake was higher for the atopy positive (0.95), compared with the atopy negative group (0.93) ($p=0.04$). The adjusted ratio of n6:n3 showed to have detrimental association with asthma (OR= 1.072, 95% CI= 1.009–1.139) and wheezing episodes (OR= 1.064, 95% CI= 1.007–1.124). This means that for every 1 level increase in the ratio of n6:n3 there was a 7.2% and 6.4% increase in the risk of developing asthma and wheezing episodes (Tables 24 and 26).

This is consistent with the literature where it is shown that an increase in the ratio of n6:n3 is associated with an increase in the risk of asthma and significant evidence is found for a modulatory effect of the dietary n-6: n-3 fatty acid ratio on the presence of asthma in adolescents (Oddy et al. 2004a; Simopoulos 2002; Strachan 1999).

The next section explores more comprehensive analysis on the relationship between red blood cell membrane n-3, n-6 and total fatty acids contents and asthmatic symptoms.

3.4 THE RELATIONSHIP BETWEEN RED BLOOD CELL MEMBRANE FATTY ACIDS CONTENTS AND ASTHMATIC SYMPTOMS -STUDY ONE

The second part of Study One was dedicated to studying the relationship between red blood cell membrane fatty acids content and asthmatic symptoms.

Analysing the effects of mean red blood cell fatty acids content on the number of times wheezed in the last 12 months revealed that none of the n-6 fatty acids had any effects on wheeze frequency. However, significant associations were found between wheeze and total n-3 fatty acids. The highest level of α -linolenic (18:3n3) acid was observed in adolescents who had the highest number of times wheezed in the last 12 months. The mean level of α -linolenic acid for adolescents with no wheeze (n=1315) was 0.22 mmol/L while for those wheezing more than 12 times within the last year (n=20) was 1.86 times higher at 0.41 mmol/L (p=0.002) (Table 14). Therefore, those adolescents who had the highest number of wheezing episodes in the last year had 0.19 mmol/L more α -linolenic acid in their red blood cells compared with those who had no wheezing episodes in the last year (p=0.002), 0.20 mmol/L more α -linolenic acid than those with 1 to 2 episodes of wheezing in the last year (0.21 mmol/L) (p=0.001) and 0.20 mmol/L more α -linolenic acid in their RBC compared to those who had 3 to 12 wheezing episodes in the last year (p=0.003). Those adolescents who had the highest levels of α -linolenic acid in their red blood cells also had the highest number of wheezing episodes compared to the other groups.

It has been shown that in infants who were born to mothers with no allergy (low-risk infants), a higher intake of α -linolenic acid (18:3n3) may increase the risk of sensitization (defined as a titer of IgE higher than 0.35 IU/mL to an allergen) (Wijga et al. 2006). Therefore, the highest level of α -linolenic acid may have resulted in a higher titer of IgE and hence the highest number of wheezing episodes was observed in this group.

There was also a significant difference between red blood cell docosapentaenoic acid (22:5n3) levels for the group that had more than 12 wheezing episodes in the last

year compared with the group with no wheezing episodes in the last year (Table 14). The highest level of docosapentaenoic acid was observed in those who had the highest number of times wheezed in the last 12 months (5.37 mmol/L) compared with 4.29 mmol/L for those with no wheeze ($p=0.035$). Therefore, adolescents who had the highest number of wheezes in the last year had 1.07 mmol/L more docosapentaenoic acid in their red blood cell membrane compared to those who had no episodes of wheezing in the last year ($p=0.035$).

There were also significant differences between current asthma, current wheeze and BHR and the lowest and the highest of the quintiles of red blood cell levels of docosapentaenoic acid. For adolescents with current asthma, current wheeze and BHR the lowest quintile of docosapentaenoic acid had the lowest of the sample population while the highest quintile consisted of highest percentage of the group (Table 14).

The mean level of red blood cell total n-3 was also significantly higher for those with the highest wheeze number compared with those with no wheeze. The mean level of total n-3 for cases with more than 12 wheezing episodes was 11.36 mmol/L compared with 10.07 mmol/L for those with no wheeze ($p=0.036$). Therefore, our findings suggest that n-3 fatty acids do not protect against wheeze severity. This contradicts some findings in the literature (Haby et al. 2001; Mickleborough et al. 2006; Peat et al. 2004) which propose a protective role for n-3 fatty acids against asthma and asthmatic symptoms. However these findings do support those which suggest that n-3 fatty acids are not associated with a reduced risk of asthma (Almqvist et al. 2007; Woods et al. 2004).

The level of red blood cell α -linolenic acid (18:3n3) was shown to be significantly higher in atopy positive (0.219 mmol/L) compared with atopy negative adolescents (0.190 mmol/L) (Table 27). When Pearson chi square of association in univariate analysis was performed to assess the differences in individual fatty acid content and the prevalence of asthmatic outcomes we found that for α -linolenic acid in atopy positive individuals ($n=584$), there was a significant difference between the lowest and the highest quintile groups. There were more atopy positive adolescents in the highest compared with the lowest α -linolenic acid quintile ($p=0.043$) (Table 15).

Therefore, we concluded that the levels of α -linolenic acid in red blood cell may be associated with atopy. No associations were found between the other outcomes (current asthma, current wheeze, BHR) and the level of α -linolenic acid in red blood cells.

In one study in mice it was shown that α -linolenic rich oils lower serum lipids and ovalbumin-specific IgG1, but increases total IgE levels (Chang, Chen & Lin 2009). This agrees with our finding in humans that the highest level of red blood cell α -linolenic acid (18:3n3) was observed in the adolescents who had the highest number of times (>12) wheezing episodes in the last 12 months. The mechanism by which α -linolenic acid increases total IgE levels is not fully understood. It is suggested however, that the modulation of T_H1/T_H2 antibody levels via isotype switching of B cells does not occur during the process of elevation of IgE by α -linolenic (Chang, Chen & Lin 2009).

Atopy also was associated with the level of parinaric acid (18:4n3) (p=0.016) (Table 11) but no associations were found between the other outcomes (current asthma, current wheeze, and BHR) and the level of parinaric acid in red blood cell.

BHR was shown to be strongly associated with the level of eicosapentaenoic acid (20:5n3) in red blood cell, as p=0.007 (Table 17). The lowest number of BHR positive adolescents was observed in the second quintile. This could indicate a direct association between the level of RBC eicosapentaenoic acid and the incidence of BHR but there was no significant trend found in rising quintiles. Therefore our finding on eicosapentaenoic acid does not confirm the hypothesis that polyunsaturated fatty acids such as eicosapentaenoic acid may be beneficial to the asthmatic condition (Nauta et al. 2008).

In crude analysis we observed that, with the exception of docosapentaenoic acid (22:5n3) in red blood cell no other fatty acids (linoleic acid, α -linolenic acid, parinaric acid, arachidonic acid, eicosapentaenoic acid, docosatetraenoic acid and docosahexaenoic acid) had a significant impact on the severity of asthma (Table 32). We showed a mean level of docosapentaenoic acid in red blood cells of adolescents with no asthma to be significantly lower compared to severe asthma (p=0.027).

These findings are in contradiction with the literature as there is much evidence proposing a protective role for n-3 fatty acids on asthma (Haby et al. 2001; Mickleborough et al. 2006; Oddy et al. 2004b; Peat et al. 2004). Although our crude analysis suggested that the higher level of docosapentaenoic acid (DPA) the higher the risk of having severe asthma (Figure 12) after controlling for potential confounders (age, sex, BMI and total energy intake) no significant roles between the concentration of DPA and asthmatic symptoms was observed (Tables: 24, 26, 28 and 30).

After adjusting for confounders, the only fatty acid that showed a significant association on one of the asthmatic symptoms (BHR) was docosahexaenoic acid (22:6n3) (DHA). Higher levels of DHA in RBC membrane showed protective effects on BHR (adjusted OR= 0.814, 95% CI= 0.669–0.989) (Table 30). Therefore, for every 1 mmol/L increased level of docosahexaenoic acid in RBC membrane there was an 18.6% reduction in the risk of having BHR ($1 - 0.814 \text{ (OR)} = 0.186 \times 100 = 18.6\%$).

There is evidence which suggests that cells may become more susceptible to oxidative injury when they are exposed to elevated amounts of polyunsaturated fatty acids due to the peroxidation of fatty acids (North et al. 1994). The peroxidation rate of the polyunsaturated fatty acids can be lowered in the presence of an optimum concentration of antioxidant vitamins (Van den Berg et al. 1990). The effect of vitamin C on the peroxidation of parinaric acid in red blood cell membranes was shown to be highly dependent on the concentration of the vitamin (Drummen et al. 2004). Vitamin E is shown to have a concentration-dependent protection of parinaric acid against peroxidation, while the protective effect of the combination of vitamins E plus C exceeds the sum of their individual effects (Van den Berg et al. 1990).

No evidence was observed for docosapentaenoic acid to be a risk factor for mild or moderate asthma. There are a few studies which suggest that n-3 fatty acids are not associated with a reduced risk of asthma (Almqvist et al. 2007; Woods et al. 2004) and our findings support this.

There was no evidence to suggest any associations between the level of arachidonic acid (20:4n6) in red blood cells and any of the outcomes of interest (current asthma, current wheeze, atopy and BHR).

Our data suggest that consumption of fish did not protect against asthma severity. The severe asthma group (n=33) had 20.08 g/day higher mean intake of steamed/grilled fish intake compared with the group with moderate asthma (n=58) (Figure 11). After controlling for confounders, we found no associations between consumption of fish and asthmatic symptoms (Tables 6-12).

3.5 DISCUSSION ON STUDY ONE RESULTS

Our data demonstrated a small protective association (0.3%) between vitamin C consumption and asthma. No protective association was found between the other antioxidants and asthmatic symptoms. The only fruit that showed a protective association (1.2%) on atopy was berry. Berry fruits are rich sources of vitamin C and their effect on atopy could be attributed to their high vitamin C content. However, other fruits (such as citrus fruits), which are also known to have high vitamin C content were shown to have no effects on the asthmatic symptoms. Berry fruits are a rich source of anthocyanins which are natural pigments that belong to the flavonoid family and are widely distributed in the human diet in foods such as beans, fruits, vegetables, and red wines (Park et al. 2007).

There are 27 known anthocyanidins (anthocyanins without sugar groups) present in nature, however, only six (cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin) are commonly found in berries (Duan et al. 2007). The major anthocyanins found in berries are identified as cyanidin 3-O-glucoside, delphinidin 3-O-glucoside and cyanidin 3-O-arabinoside. Antioxidative properties of anthocyanins in berries arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol-derived radicals to stabilize and delocalize the unpaired electron, and also from their ability to chelate transition metal ions (Duan et al. 2007).

Anthocyanins have been shown previously to reduce the levels of inflammatory mediators in a lung inflammatory disease model in rats (Rossi et al. 2003). A recent study suggests that anthocyanins inhibit airway inflammation and hyperresponsiveness in asthma (Park et al. 2007). It is proposed that anthocyanins may undermine the development of asthma by down-regulating T-helper type 2 (TH₂) cytokines, and cyclooxygenase COX-2 mRNA (Jeon et al. 2007; Park et al. 2007). Therefore, the protective role of berry fruits on wheezing episodes observed here could be due to the presence of anthocyanins.

Therefore, the reduction in the risk of developing atopy associated with higher consumptions of berry fruits could be due to a higher intake of anthocyanins in this group. None of the other antioxidants (vitamins A, E, magnesium, zinc and β -carotene) in our study demonstrated a protective association with asthmatic symptoms. Our preliminary results showed that adolescents with the most severe asthma had the highest intake of combined vegetables and fruits and vitamin A. Further investigations however, revealed that the association between the consumptions of these nutrients was due to the interference of confounders and therefore after introduction of the confounders the effect was not significant.

In animal and in-vitro models many studies have implicated mast cells in the full expression of asthma (Matsubara et al. 2006; Metz, Siebenhaar & Maurer 2008); although other studies have shown that clinical features of asthma can develop in the absence of mast cells or IgE (Hamelmann & Gelfand 1999; Kraneveld et al. 2005). In asthmatic people, mast cells migrate into the airway epithelium, the airway mucous and smooth muscle glands (Nauta et al. 2008). The number of mast cells infiltrated in the airway smooth muscle bundles has been shown to correlate significantly with BHR in asthmatics, implicating their importance for the pathophysiology of asthma while virtually no mast cells were found in these regions in non-asthmatics (Bradding & Brightling 2007). It is proposed that foods and nutrients such as antioxidants (by inhibiting TNF- α) and n-3 fatty acids (by inhibiting APC function) can interrupt the infiltration of mast cells in the airway smooth muscle (van de Laar & van der Korst 1992).

While the average daily intake of n-3 fatty acid in our cohort was within the RDI level (1.2-2.4 g/day), the average daily intake for n-6 and the ratio of n6:n3 were between 4 to 5 times higher than the RDI level (Table 4). The ratio of n6:n3 was higher in atopy positive teenagers compared to atopy negative individuals. This finding is in accordance with the literature, as it is proposed that increases in intake of foods containing n-6 fatty acids can increase the synthesis of prostaglandin E2, therefore increasing the risk of inflammation, which may increase the risk of asthma (Black & Sharpe 1997; Bolte et al. 2006; Gold et al. 2006; Haby et al. 2001; Marangoni et al. 2007).

Our initial investigations indicated that higher levels of docosapentaenoic acid (DPA) (22:5n3) in red blood cells were positively associated with severe asthma, atopy, wheeze and increasing number of wheezing episodes. However, after further analyses and controlling for confounders it was revealed that DPA did not have a significant association with the asthmatic symptoms of interest. These findings contradict those of Reichardt et al (2004) who proposed that a lower level of docosapentaenoic acid (22:5n3) is associated with elevated total serum IgE ($p < 0.05$) in one year old children although our participants were older.

Preliminary results also indicated that higher levels of α -linolenic acid (18:3n3) and parinaric acid (18:4n3) in red blood cells were positively associated with atopy. Alpha-linolenic acid was also positively associated with increasing number of wheezing episodes. After adjusting for confounders and performing further analyses it was revealed that α -linolenic acid and parinaric acid did not play a significant role in manifestation of the asthmatic symptoms.

Our initial results showed that an increase in the total level of red blood cell n-3 fatty acids was associated with an increase in the number of the wheezing episodes and thus asthma severity. There is one study which demonstrates that frequency of fish intake is positively related to the prevalence of asthma during childhood (Takemura et al. 2002). There are however other studies which propose that increased consumption of fish is associated with a reduced risk of asthma (Peat, Salome & Woolcock 1992). Other studies suggest that using fish oil supplements makes no

clinical improvement in patients with asthma and that despite reduced TNF- α production; no effects on the clinical severity of asthma have been observed (Anticevich et al. 1995; Hodge et al. 1998). After performing further analyses and controlling for the effects of confounders it was revealed that total level of n-3 fatty acids did not have a significant role on asthmatic symptoms.

A decrease in docosatetraenoic acid (22:4 n6) may be associated with an increase in the amount of docosahexaenoic acid (22:6n3) (Chavali, Zhong & Forse 1998). Increased level of docosahexaenoic acid subsequently results in more binding of this fatty acid to cyclo-oxygenase molecules and, consequently, reduces the formation of pro-inflammatory prostaglandin E2 and increases formation of less inflammatory prostaglandin E1 and E3. This agrees with our findings that docosahexaenoic acid might play a protective role against BHR.

3.6 SUMMARY OF DISCUSSION OF STUDY ONE RESULTS

We found a protective association between the consumption berry of fruits and atopy. We propose that this is due to the high content of anthocyanins in these fruits as the role of this antioxidant against asthmatic inflammation has been documented (Park et al. 2007). We also found a protective association between vitamin C consumption and asthma. This may be due to the suppression of NF- κ B activity by vitamin C (Sackesen et al. 2008). No other associations were found between the other antioxidants and asthmatic symptoms.

We observed a protective association for docosahexaenoic acid (22:6n3) on BHR. This could be due to the proposed role of this fatty acid for binding cyclo-oxygenase molecules and, consequently, reducing the formation of pro-inflammatory prostaglandin E2 and increase formation of less inflammatory prostaglandin E1 and E3 (Chavali, Zhong & Forse 1998).

We showed that the ratio of n-6:n-3 was higher in asthmatics and wheezers compared with non-asthmatics and non-wheezing individuals. This may be due to an increase in the synthesis of prostaglandin E2 as a result of the higher intake of foods rich in n-

6 fatty acids (Black & Sharpe 1997; Bolte et al. 2006; Gold et al. 2006; Haby et al. 2001; Marangoni et al. 2007).

CHAPTER FOUR

STUDY TWO RESULTS

Results from Study Two are reported in this chapter. This study was conducted to assess the current asthmatic symptoms and dietary intake (fatty acid and antioxidant nutrients) of previously selected cases and controls (Oddy et al. 2004a) and re-assess their asthmatic and dietary status six years later.

4.1 THE RESULTS OF STUDY TWO:

Study Two was a longitudinal analysis from a subset of the overall cross-sectional Study One. In this study participants of the previous nested case control study at the age of eight (n=335), were followed up six years later at 14 years of age (n=242) (Oddy et al. 2004a). Cases of asthma and controls were identified from the six year follow-up of the nested case-control study. The diagnosis of current asthma was confirmed by diagnosis of asthma by a doctor and wheeze in the last year.

4.1.1 The population

The reporting of asthma in children at both eight and 14 years were combined into one categorical variable and a new variable was created with four groups. Group 1: no asthma at either ages of eight and 14, group 2: asthma only at the age of eight but not at 14, group 3: asthma only at the age of 14 but not at eight and group 4: asthma at both ages of eight and 14 years (summarised in Table 33). One way analysis of variance was performed for these variables and the different nutrients of interest (Tables 34 & 35).

Table 33: Occurrence or reporting of asthma at eight and 14 years

| Groups | n | Asthma at 8 years of age | Asthma at 14 years of age |
|---------------|----------|---------------------------------|----------------------------------|
| 1 | 123 | No | No |
| 2 | 68 | Yes | No |
| 3 | 6* | No | Yes |
| 4 | 45 | Yes | Yes |
| Total | 242 | | |

* Very small “n” in this group is acknowledged therefore, results related to this group may be limited.

4.1.2 The effects of antioxidant and fruit intake on asthma history

In crude analyses we found that there were significant differences ($p < 0.05$) in the mean intake of vitamin A between those who had asthma at eight years only ($n=68$) and those who did not have asthma at that age but developed asthma later at the age of 14 ($n=6$). The mean intake of vitamin A was 772.77 $\mu\text{g/day}$ higher for those who developed asthma at the age of 14 compared to those who had asthma at the age of eight years only (1246.41 $\mu\text{g/day}$) ($p=0.019$) (Figure 17) and also higher (789.49 $\mu\text{g/day}$) than the group who did not have asthma at either eight or 14 (1229.68 $\mu\text{g/day}$) ($p=0.014$) ($n=123$).

As stated in section 3.2.2.1, CI for the means of two factor levels can overlap, but the difference between them can be significant at the $p < 0.05$. Hence, figures 17-23 demonstrate significant differences in the intake of a variety of foods and nutrients in asthmatic groups despite the presence of overlaps between the CI.

The highest mean intake of vitamin C was 234.76 mg/day in adolescents with asthma only at 14 years ($n=6$) whereas the lowest mean intake of vitamin C was 192.42 mg/day in those who had asthma at eight years only ($n=68$). Similarly, the highest intake of β -carotene was 3876.65 $\mu\text{g/day}$ in adolescents who had asthma at 14 and

the lowest intake was 2166.02 µg/day in those who had asthma at the age of eight (n=68) although this difference was not significant.

The lowest intake of vitamin E was 8.35 mg/day in those who never had asthma (n=123) and the highest intake was in the group who had asthma at both eight and 14 (9.55 µg/day) (n=45) although, this difference was not statistically significant (Table 25).

The lowest intake of magnesium was 304.02 mg/day and was recorded for the group who had asthma at the age of eight years (n=68). The highest intake of magnesium was 339.88 mg/day and was seen in adolescents with asthma at 14 only (n=6). This difference was not statistically significant.

The lowest mean intake of zinc was 12.91 mg/day and was observed in the group with no asthma at eight or 14 (n=123). The highest mean intake of zinc was 14.08 mg/day and was observed in the adolescents with asthma at 14 only (n=6). The latter also showed the highest variation in their zinc intake, although none of these differences recorded significance.

The association between dietary fruits and vegetables with asthma was assessed in detail using subgroups of fruit: citrus fruits, berry fruits, pome fruits such as apples and pears, stone fruits and a combination of all these fruits.

There was a large difference in the mean intake of berry fruits across the four groups (Table 34). The lowest intake of berry fruit intake was 5.31 g/day in adolescents with asthma at 14 years only (n=6) compared with the highest intake of 12.40 g/day in those reporting asthma at eight years only (n=68). However, the differences in the intake of berry fruits were not significant. There were also large variations in the intake of citrus fruits. The highest intake of citrus fruits was 61.02 g/day and was recorded for those with asthma at 14 only and the lowest was 25.73 g/day recorded for those with no asthma.

Those participants who had asthma at eight years of age only, had the highest mean intake of pome fruit with 120.16 g/day. The pome fruit intake was considerably higher than the group who had an asthma history at the age of 14 only with 33.43 g/day. Despite the large magnitude of difference in pome fruits intake between these two groups, the difference was not significant due to the small sample size in the group with asthma at 14 only (n=6).

The mean intake of tropical fruit was very similar for the group who had asthma at the age of eight only (n=68) with a mean intake of 76.79 g/day and those with asthma at 14 years of age only (n=6) with 71.81 g/day. The former also had the highest mean intake of tropical fruits compared with the other groups. Those who had asthma at both ages of eight and 14 had the lowest intake of tropical fruits with 46.23 g/day.

The average daily consumption of stone fruit was significantly higher for those children who had asthma at eight years only (n=68) with 44.76 g/day compared with those who did not have asthma at either age with an average daily consumption of 28.90 g/day (n=123). Therefore, the mean difference in stone fruit intake was 15.86 g/day more for those with asthma at the age of only eight compared with those who did not have asthma at either ages ($p=0.025$) (Figure 18). The mean intake of stone fruit was also higher for the children with asthma at eight years only (n=68) with 44.76 g/day compared with adolescents who had asthma at both ages of eight and 14, (n=45) with an average intake of 20.27 g/day (Figure 18 and Table 34).

Intake of all four different fruit groups (pome, berry, citrus and stone fruits) were combined to produce a new variable called combined fruits. There were significant differences in the mean intake of combined fruits for children with asthma at the age of eight (n=68) as they had 68.26 g/day higher intake than those with no asthma (n=123) ($p=0.012$) (Figure 18). The mean daily intake of combined fruits was significantly lower ($p=0.035$) in adolescents who had asthma at both ages of eight and 14 (n=45) with an average daily intake of 143.84 g/day compared with those who only had asthma at eight years of age (n=68) with 216.48 g/day (Figure 19 and Table 34).

The intake of 14 different vegetables (carrots, turnip, broad beans, green beans, capsicum, cabbages, green peas, Brussels sprouts, spinach, broccoli, cauliflower, pumpkins, sweet corn, and zucchini) were also combined to make a unique combination of vegetables ingested during both summer and winter. Analysis of variance showed no significant differences between the average daily intakes of vegetables with asthma outcome for any of the groups (data not shown).

Table 34: Analysis of variance of different antioxidants and fruits intake with history of asthma.

| History of asthma | Mean vitamin A intake µg /day (±SEM) | Mean vitamin C intake mg/day (±SEM) | Mean vitamin E intake mg/day (±SEM) | Mean β – carotene intake µg/day (±SEM) | Mean magnesium intake mg/day (±SEM) | Mean zinc intake mg/day (±SEM) | Mean of berry fruits intake g/day (±SEM) | Mean of citrus fruits intake g/day (±SEM) | Mean of pome fruits intake g/day (±SEM) | Mean of stone fruits intake g/day (±SEM) | Mean of tropical fruits intake g/day (±SEM) | Mean of combined fruits g/day (±SEM) |
|-----------------------------------|--|--|--|---|--|--|--|--|--|---|--|--|
| N | | | | | | | | | | | | |
| A Never 123 | 1229.68 (40.27) | 197.56 (9.66) | 8.35 (0.39) | 2735.10 (226.95) | 314.88 (9.05) | 12.91 (0.37) | 8.68 (1.41) | 25.73 (3.24) | 84.91 (7.65) | 28.90 (3.47) | 54.51 (5.33) | 148.22 (10.62) |
| B Asthma At 8 years only 68 | 1246.41 (69.72) | 192.42 (16.29) | 9.15 (0.56) | 2166.02 (346.84) | 304.02 (12.83) | 12.28 (0.52) | 12.40 (3.03) | 39.16 (9.00) | 120.16 (23.04) | 44.76 (7.93) | 76.79 (14.58) | 216.48 (33.29) |
| C Asthma At 14 years only 6 | 2019.18 (730.99) | 234.76 (58.76) | 9.41 (1.44) | 3878.65 (1646.97) | 339.88 (55.10) | 14.08 (2.41) | 5.31 (5.30) | 61.02 (37.86) | 33.43 (24.92) | 16.07 (7.62) | 71.81 (28.70) | 115.83 (52.62) |
| D Asthma At 8 & 14 years 45 | 1326.40 (197.73) | 173.47 (15.13) | 9.55 (0.64) | 2270.09 (288.84) | 332.24 (17.92) | 13.31 (0.68) | 8.98 (2.07) | 33.19 (14.96) | 81.40 (11.17) | 20.27 (5.26) | 46.23 (10.15) | 143.84 (21.34) |
| p-value | 0.885 (A,B) 0.014 (A,C) 0.079 (A,D) 0.019 (B,C) 0.138 (B,D) 0.098 (C,D) | 0.769 (A,B) 0.441 (A,C) 0.232 (A,D) 0.390 (B,C) 0.393 (B,D) 0.223 (C,D) | 0.229 (A,B) 0.561 (A,C) 0.116 (A,D) 0.886 (B,C) 0.631 (B,D) 0.942 (C,D) | 0.662 (A,B) 0.229(A,C) 0.298 (A,D) 0.179 (B,C) 0.567 (B,D) 0.108 (C,D) | 0.501 (A,B) 0.575 (A,C) 0.351 (A,D) 0.431 (B,C) 0.170 (B,D) 0.869 (C,D) | 0.324 (A,B) 0.516 (A,C) 0.595 (A,D) 0.324 (B,C) 0.209 (B,D) 0.680 (C,D) | 0.183 (A,B) 0.612 (A,C) 0.925 (A,D) 0.301 (B,C) 0.333 (B,D) 0.600 (C,D) | 0.181 (A,B) 0.142 (A,C) 0.512 (A,D) 0.375 (B,C) 0.638 (B,D) 0.270 (C,D) | 0.055 (A,B) 0.239 (A,C) 0.865 (A,D) 0.054 (B,C) 0.095 (B,D) 0.297 (C,D) | 0.025 (A,B) 0.447 (A,C) 0.282 (A,D) 0.099 (B,C) 0.007(B,D) 0.813 (C,D) | 0.074 (A,B) 0.560 (A,C) 0.558 (A,D) 0.870 (B,C) 0.054 (B,D) 0.413 (C,D) | 0.012 (A,B) 0.616 (A,C) 0.886 (A,D) 0.130 (B,C) 0.035 (B,D) 0.680 (C,D) |

Fisher's LSD p-value was used to compare between two groups means after ANOVA was performed.

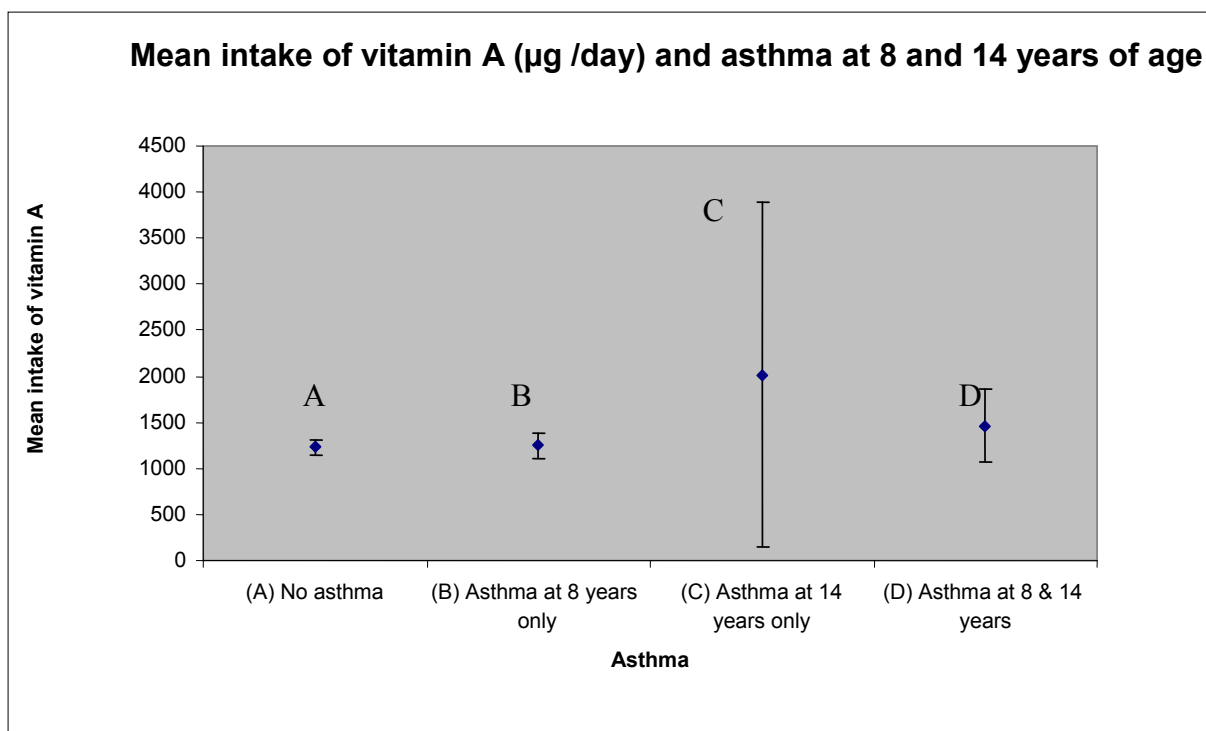


Figure 17 - Mean intake of vitamin A ($\mu\text{g/day}$) (\pm 95% confidence intervals) and asthma at eight and 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A & C ($\alpha=0.014$) and groups 1 & 2 ($\alpha=0.019$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.885 |
| A, C | 0.014 |
| A, D | 0.079 |
| B, C | 0.019 |
| B, D | 0.138 |
| C, D | 0.098 |

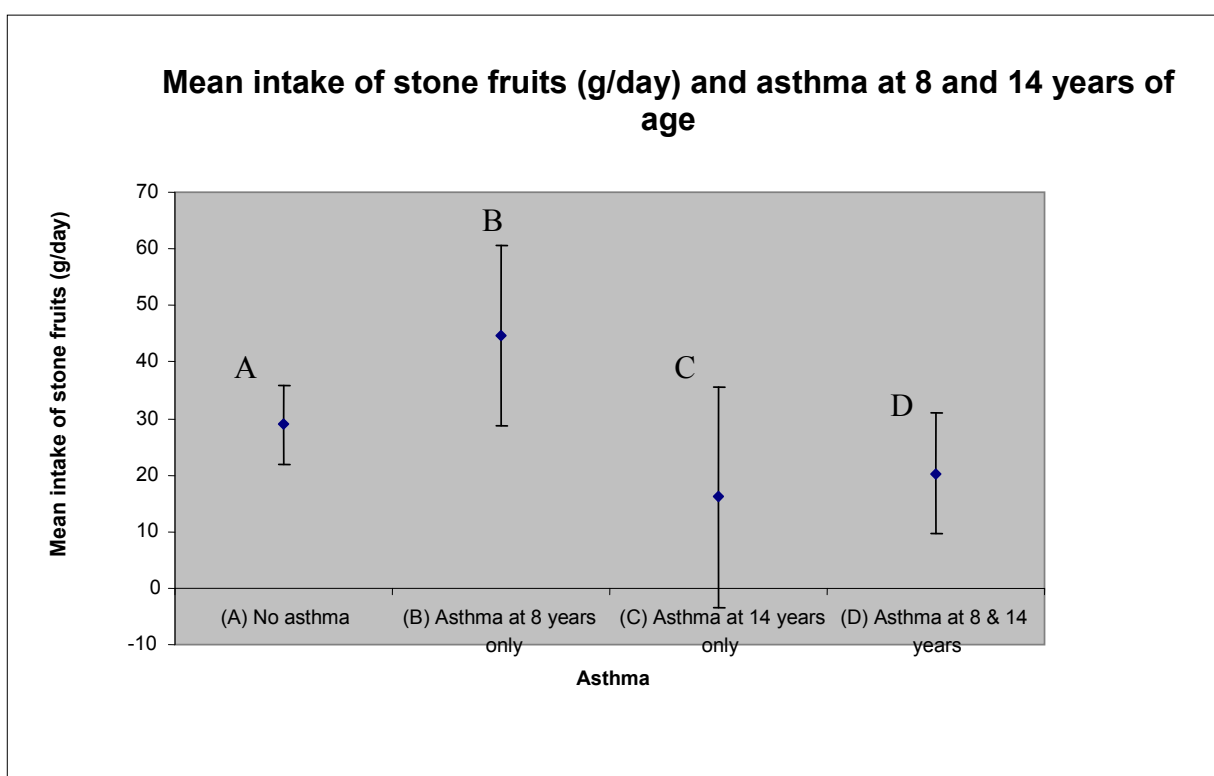


Figure 18 – Mean intake of stone fruits (g/day) (+/- 95% confidence intervals) and asthma at eight and 14 years of age.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, B ($\alpha=0.025$) and groups B, D ($\alpha=0.007$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.025 |
| A, C | 0.447 |
| A, D | 0.282 |
| B, C | 0.099 |
| B, D | 0.007 |
| C, D | 0.813 |

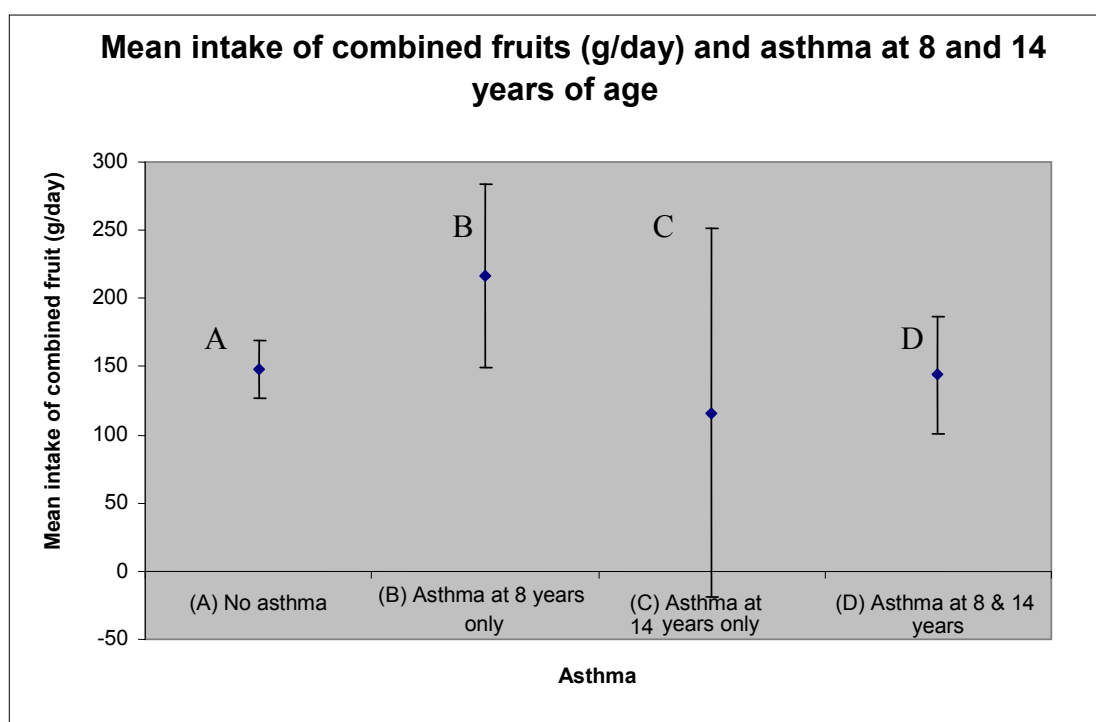


Figure 19 – Mean intake of combined fruits (g/day) (mean +/- 95% confidence intervals) and asthma at eight and 14 years of age for asthma.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, B ($\alpha=0.012$) and groups B, D ($\alpha=0.035$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.012 |
| A, C | 0.616 |
| A, D | 0.886 |
| B, C | 0.130 |
| B, D | 0.035 |
| C, D | 0.680 |

In summary, in crude analysis, the only antioxidant nutrients and foods which showed significant associations with asthma history were vitamin A, stone fruits and combined fruits. The mean intake of vitamin A was higher in adolescents who had asthma at the age of 14 compared to those who did not have any history of asthma earlier at the age of eight. Lowest consumption of stone fruits was observed in adolescents who had a history of asthma at the age of 14. The mean intake of combined fruits showed that a significant reduction in the average intake of these fruits in participants from eight to 14 years resulted in the persistence of a history of asthma. However, after controlling for potential confounders (age, sex, BMI and total energy) none of the antioxidants and fruits were shown to be associated with the history of asthma (data not shown).

4.1.3 Effect of fatty acids intake on asthma history

Reported dietary intake of fatty acids obtained from the CSIRO FFQ was used for analysis in Study Two, because red blood cell fatty acid content was not available at the age of eight years and dietary fatty acid estimates were available. The dietary fatty acids that we considered for this study were five n3 fatty acids, α -linolenic acid (18:3n3), parinaric acid (18:4n3), eicosapentaenoic acid (20:5n3), docosapentaenoic acid (22:5n3) and docosahexaenoic acid (22:6n3) and three n6 fatty acids: linoleic acid (18:2n6), arachidonic acid (20:4n6) and, docosatetraenoic acid (22:4n6).

The mean intake of arachidonic acid for the group with no asthma was 13.41 g/day while the intake for the group with asthma at 14 years was 10.75 g/day, although the numbers were small in the 14 only group (n=6) (Figure 20).

There were differences ($p < 0.05$) in the mean intake of arachidonic acid, total n-3, total n-6 and the ratio of n6:n3 fatty acids between the different groups. The levels of all the individual n-3 fatty acids as well as total n-3 fatty acids were uniformly lower for the adolescents who had asthma at both ages of eight and 14 years compared to those who never had asthma (Table 35). The only significant difference in the intake of n-3 fatty acids among the four groups (never asthma, asthma at eight, asthma at 14 and asthma at eight and 14) was observed in intake of total n-3 fatty acids. The intake of total n-3 fatty acids was higher ($p = 0.009$) for those with no asthma (11.13 ± 0.25 g/day) compared to 9.82 ± 0.46 g/day for those with asthma at both eight and 14 years. The intake of total n-3 fatty acids was also higher for those with asthma at eight years only (10.96 ± 0.30 g/day) compared to the group who had asthma at both eight and 14 years (Figure 21 and Table 35).

The mean level of all the individual n-3 fatty acids was lower in the group who had asthma at both eight and 14 years but no significant differences were found (Table 35). The levels of the individual n-6 fatty acids linoleic acid (18:2n6) and docosatetraenoic acid (22:4n6) were higher for adolescents who had asthma at eight and 14 years of age compared with those who did not report asthma at either age but not significantly so (Table 35). In contrast to linoleic and docosatetraenoic acids, the

mean intake of arachidonic acid was lower for the adolescents with asthma at eight and 14 years (13.21 g/day) compared to those with no asthma (13.41 g/day) but this was not significant.

The highest intake of total n-6 fatty acids was observed in adolescents who had asthma at eight and 14 years (29.32 g/day). This intake was significantly higher compared with the total n-6 fatty acids intake of the group who had asthma only at 14 years of age (23.98 g/day). The latter had the lowest intake of the total n-6 fatty acids, and showed the most variations in the intake of the total n-6 fatty acids compared with the other groups (Figure 22). There were also significant differences in the intake of total n-6 fatty acids between the groups with no asthma at any age (29.79 g/day) compared to the group who had asthma at 14 years only (23.98 g/day) (Figure 22 and Table 35).

The mean ratio of n6:n3 was higher for the adolescents who had asthma at eight and 14 years (3.38) compared with the group who did not have asthma (2.78). The group with asthma at both ages also showed a higher ratio of n6:n3 compared with the group who had a history of asthma at 14 years only (2.14). The group with asthma at both ages also demonstrated a higher ratio of n6:n3 intake compared with those who had asthma at eight years only (2.78) (Figure 23 and Table 35).

However, after controlling for potential confounders (age, sex, BMI and total energy), only n6:n3 ratio between the non-asthmatic and the asthmatics at eight and 14 years remained significant (Table 36).

Table 35: Mean (\pm Standard Error) of dietary fatty acid intake (g/day) for different groups.

| History of asthma N | 18:3n3 Alpha-linolenic acid (ALA) (\pm SEM) | 18:4n3 Parinaric acid (\pm SEM) | 20:5n3 Eicosapent aenoic acid (EPA) (\pm SEM) | 22:5n3 Docosapent aenoic acid (DPA) (\pm SEM) | 22:6n3 Docosahexa enoic acid (DHA) (\pm SEM) | 18:2n6 Linoleic acid (\pm SEM) | 20:4n6 Arachidonic acid (AA) (\pm SEM) | 22:4n6 Docosatetra enoic acid (\pm SEM) | Total n-3 (\pm SEM) | Total n-6 (\pm SEM) | Ratio n6:n3 (\pm SEM) |
|---------------------------------------|--|--|--|--|--|--|---|--|--|--|---|
| A Never 123 | 0.28 (0.04) | 0.57 (0.03) | 0.69 (0.02) | 5.18 (0.21) | 4.41 (0.14) | 10.05 (0.15) | 13.41 (0.27) | 1.00 (0.14) | 11.13 (0.25) | 29.27 (0.44) | 2.78 (0.09) |
| B Asthma At 8 years only 68 | 0.20 (0.03) | 0.57 (0.04) | 0.68 (0.04) | 5.28 (0.29) | 4.22 (0.16) | 9.98 (0.17) | 13.07 (0.32) | 1.16 (0.22) | 10.96 (0.30) | 29.09 (0.54) | 2.78 (0.12) |
| C Asthma At 14 years only 6 | 0.31 (0.16) | 0.64 (0.09) | 0.86 (0.26) | 5.30 (0.98) | 4.38 (1.22) | 9.29 (0.31) | 10.75 (2.29) | 0.51 (0.13) | 11.50 (1.43) | 23.98 (2.80) | 2.14 (0.31) |
| D Asthma At 8 & 14 years 45 | 0.17 (0.02) | 0.52 (0.04) | 0.64 (0.05) | 4.46 (0.38) | 4.03 (0.18) | 10.19 (0.23) | 13.21 (0.48) | 1.35 (0.39) | 9.82 (0.46) | 29.32 (0.66) | 3.38 (0.35) |
| p-value | 0.180 (A,B) 0.845 (A,C) 0.105 (A,D) 0.508 (B,C) 0.661 (B,D) 0.400 (C,D) | 0.980 (A,B) 0.555 (A,C) 0.324 (A,D) 0.567 (B,C) 0.364 (B,D) 0.336 (C,D) | 0.760 (A,B) 0.173 (A,C) 0.339 (A,D) 0.149 (B,C) 0.528 (B,D) 0.091 (C,D) | 0.771 (A,B) 0.905 (A,C) 0.088 (A,D) 0.988 (B,C) 0.078 (B,D) 0.423 (C,D) | 0.040 (A,B) 0.963 (A,C) 0.165 (A,D) 0.805 (B,C) 0.518 (B,D) 0.597 (C,D) | 0.758 (A,B) 0.244 (A,C) 0.621 (A,D) 0.301 (B,C) 0.494 (B,D) 0.189 (C,D) | 0.458 (A,B) 0.039 (A,C) 0.722 (A,D) 0.077 (B,C) 0.803 (B,D) 0.066 (C,D) | 0.576 (A,B) 0.529 (A,C) 0.289 (A,D) 0.417 (B,C) 0.597 (B,D) 0.304 (C,D) | 0.682 (A,B) 0.759 (A,C) 0.009 (A,D) 0.657 (B,C) 0.040 (B,D) 0.178 (C,D) | 0.802 (A,B) 0.009 (A,C) 0.947 (A,D) 0.013 (B,C) 0.800 (B,D) 0.011(C,D) | 0.990 (A,B) 0.257 (A,C) 0.013(A,D) 0.268 (B,C) 0.025 (B,D) 0.038(C,D) |

Fisher's LSD p-value was used to compare between two groups means after ANOVA was performed.

Table 36: Association between fatty acids intake (g/day) and history of asthma after adjusting for confounders (age, sex, BMI and total energy).

| History of asthma N | 20:4n6 Arachidonic acid (AA) (±SEM) | Total n-3 (±SEM) | Total n-6 (±SEM) | Ratio n6:n3 (±SEM) |
|---------------------------------------|--|---|---|---|
| A Never 123 | 13.41 (0.27) | 11.13 (0.25) | 29.27 (0.44) | 2.78 (0.09) |
| B Asthma At 8 years only 68 | 13.07 (0.32) | 10.96 (0.30) | 29.09 (0.54) | 2.78 (0.12) |
| C Asthma At 14 years only 6 | 10.75 (2.29) | 11.50 (1.43) | 23.98 (2.80) | 2.14 (0.31) |
| D Asthma At 8 & 14 years 45 | 13.21 (0.48) | 9.82 (0.46) | 29.32 (0.66) | 3.38 (0.35) |
| p-value | 1.000 (A,B) 0.330 (A,C) 1.000 (A,D) 0.627 (B,C) 1.000 (B,D) 0.510 (C,D) | 1.000 (A,B) 1.000 (A,C) 0.094(A,D) 1.000 (B,C) 0.204 (B,D) 1.000 (C,D) | 1.000 (A,B) 0.907 (A,C) 1.000 (A,D) 0.136 (B,C) 1.000 (B,D) 0.112(C,D) | 1.000 (A,B) 1.000 (A,C) 0.030(A,D) 1.000 (B,C) 0.173 (B,D) 0.823(C,D) |

Significance levels were adjusted for multiple comparisons by using the Bonferroni method.

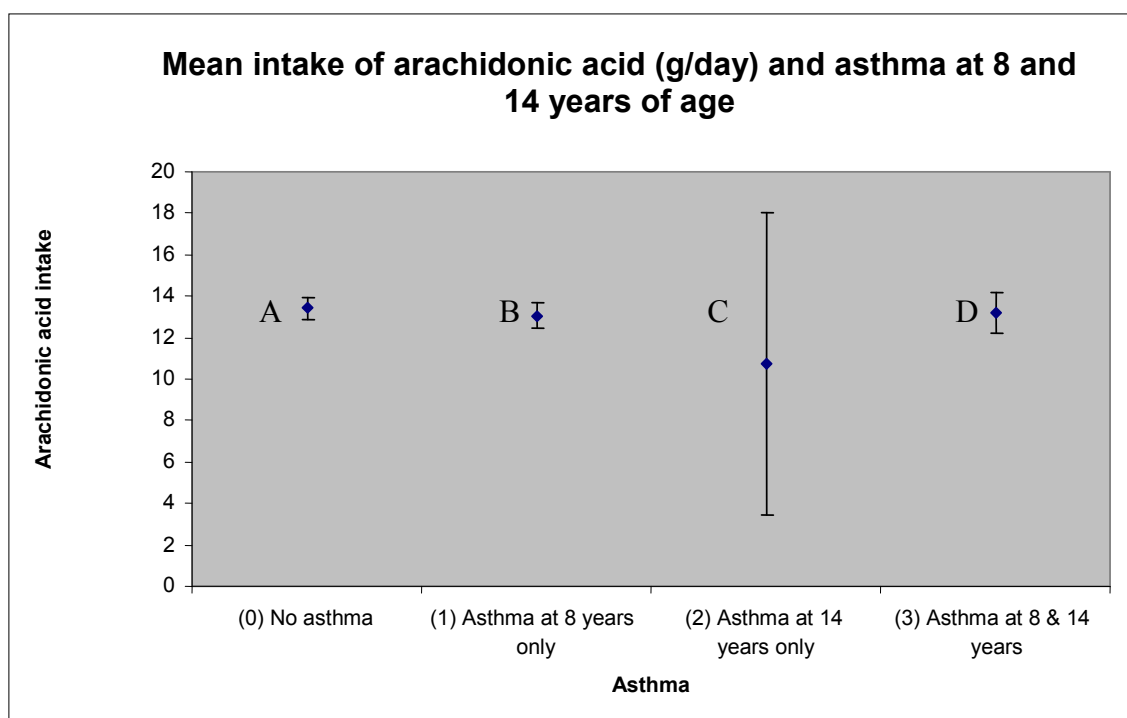


Figure 20 – Mean intake of arachidonic acid (20:4n6) (mean \pm 95% confidence intervals) at eight and 14 years of age for asthma.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, C ($p=0.039$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.458 |
| A, C | 0.039 |
| A, D | 0.722 |
| B, C | 0.077 |
| B, D | 0.803 |
| C, D | 0.066 |

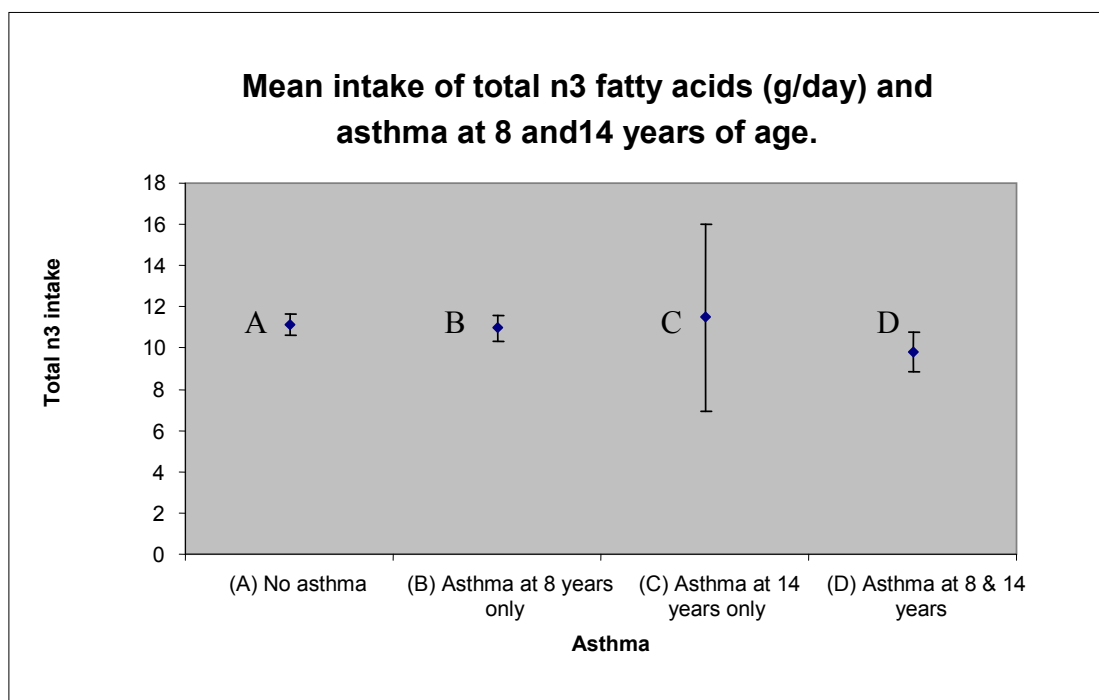


Figure 21 - Mean intake of total n3 fatty acids (mean +/- 95% confidence intervals) at eight and 14 years of age for asthma.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, D ($p=0.009$) and groups B, D ($p=0.04$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.682 |
| A, C | 0.759 |
| A, D | 0.009 |
| B, C | 0.657 |
| B, D | 0.040 |
| C, D | 0.178 |

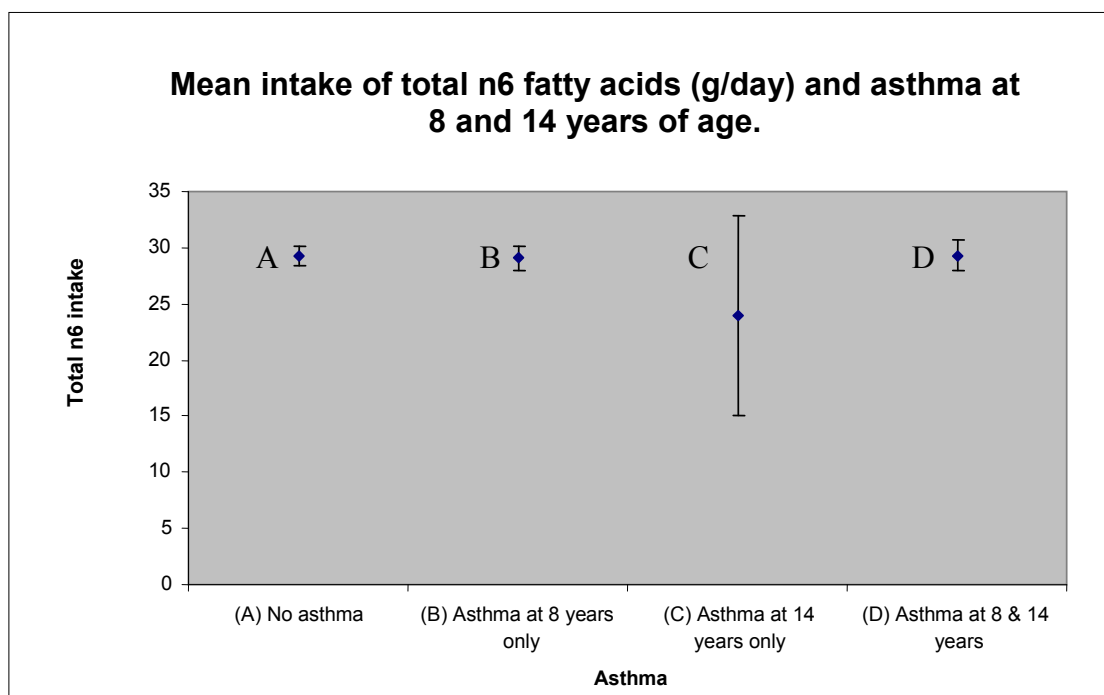


Figure 22 – Mean intake of total n-6 fatty acids (mean +/- 95% confidence intervals) at eight and 14 years of age for asthma.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, C (p=0.009), groups B, C (p=0.013) and groups C, D (p=0.011).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.802 |
| A, C | 0.009 |
| A, D | 0.947 |
| B, C | 0.013 |
| B, D | 0.800 |
| C, D | 0.011 |

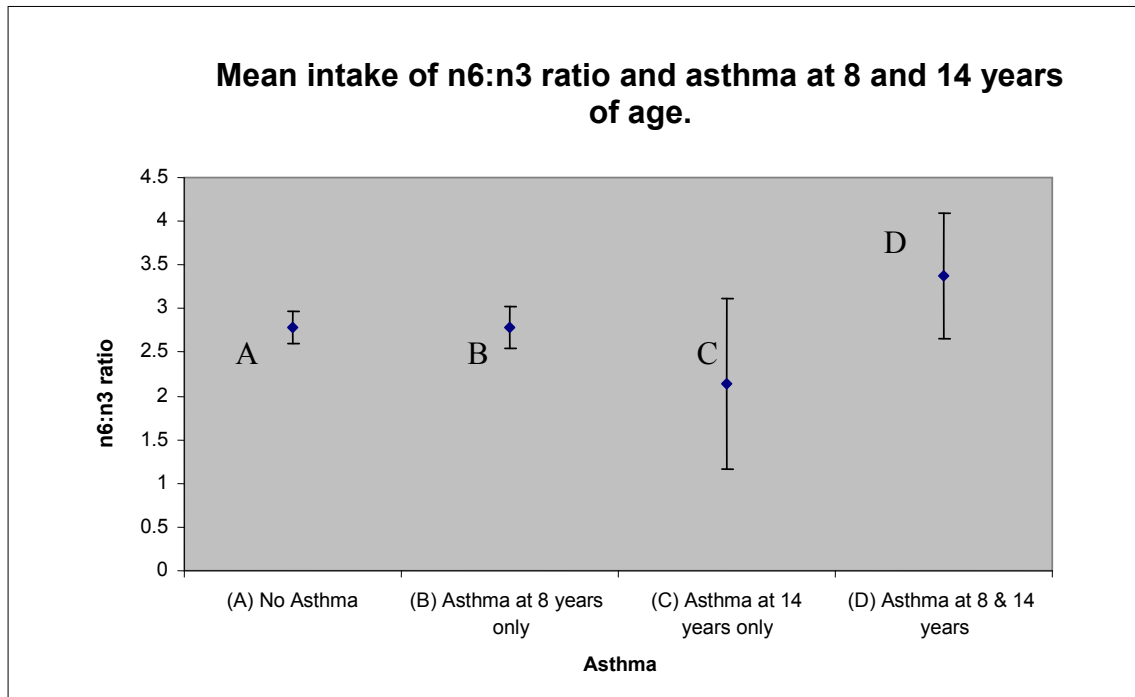


Figure 23 – Mean intake of the ratio of n6:n3 fatty acids (mean +/- 95% confidence intervals) at eight and 14 years of age for asthma.

Fisher's LSD p-value was used to compare between the two groups means after ANOVA was performed. Significant differences between groups A, D ($p=0.013$), groups B, D ($p=0.025$) and groups C, D ($p=0.038$).

P-value between groups:

| | |
|-------------|--------------|
| A, B | 0.990 |
| A, C | 0.257 |
| A, D | 0.013 |
| B, C | 0.268 |
| B, D | 0.025 |
| C, D | 0.038 |

In summary in crude analysis significant associations were observed between the intake of total n-3, total n-6 and the ratio of n6:n3 fatty acids with asthma. The only individual fatty acid that demonstrated a significant role in the history of asthma was the n-6 fatty acid, arachidonic acid, where intake was lower in adolescents with asthma at only 14 years of age compared with those who did not have asthma at any age.

4.2 *Effect of antioxidants intake on asthma history*

The first part of Study Two was devoted to investigating the effects of antioxidant intake on asthma history. After adjusting for potential confounders (age, sex, BMI and total energy) none of the antioxidant nutrients and foods showed significant effects on asthma history.

4.2.1 *Effect of fatty acids intake on asthma*

The second part of Study Two was dedicated to investigating the effects of fatty acid intake on asthma history. After controlling for potential confounders significant association in the ratio of n6:n3 fatty acids between the non-asthmatics and asthmatics at eight and 14 years was found.

4.3 *DISCUSSION*

The results of Study Two will be discussed in the following sections.

4.3.1 *Antioxidants and fruits*

Our results did not demonstrate a protective association between any of the antioxidants and asthma at either age of eight or 14.

In crude analyses we found that, the mean intake of vitamin A was higher in adolescents who had asthma at the age of 14 compared to those who did not have any history of asthma earlier at the age of eight. This finding contradicts the hypothesis that vitamin A as a part of the antioxidant system, has a protective role against asthma (de Luis et al. 2005; Hatch 1995; McKeever & Britton 2004; Soutar, Seaton & Brown 1997), and those studies which associate high serum levels of vitamin A with an increased risk of IgE sensitization (Kull et al. 2006). After controlling for

confounders, we found no associations between the intake of vitamin A and the history of asthma.

Important differences were observed in crude analyses in the mean intake of combined fruits and the history of asthma. Comparison of the mean intake of these fruits showed that a significant reduction in the average intake of fruits in participants from eight to 14 years resulted in the persistence of a history of asthma. However, after adjusting for confounders the associations between the intake of these fruits and asthma was shown as non-significant.

Consumption of none of the fruit groups (berry, citrus, pome, stone, tropical and combination of all fruits) showed a protective association for asthma. We did not observe any significance associations between consumption of berry, citrus and pome fruits and asthma. However, in crude analysis, we found significant differences in the consumption of stone fruits and combined fruits and their associations with asthma in different groups. Our crude analysis suggests that a lower consumption of stone fruits could contribute to the persistence of asthmatic symptoms later in life. It showed that the lowest consumption of stone fruits was observed in adolescents who had a history of asthma at the age of 14 (16.07 g/day) followed by those who had asthma at both ages of eight and 14 (20.27 g/day). However, the conclusion that consumption of stone fruits can offer protective roles against asthmatic symptoms cannot be conclusive as when we controlled for confounders, none of these associations were shown to be significant.

The average intake of vitamin C, vitamin E, β -carotene, manganese, zinc, berry fruits, citrus, pome and tropical fruits did not show any significant effect on asthma history. Thus, our study does not agree with those that propose protective roles for these vitamins micronutrients or fruits against asthma (Soutar, Seaton & Brown 1997; Zalewski et al. 2005). Since fresh fruits and vegetables are rich sources of antioxidants, these findings contradict the hypothesis that dietary antioxidant intakes may modify the risk of developing asthma (de Luis et al. 2005; Martindale et al. 2005; Soutar, Seaton & Brown 1997) as no evidence of any association was found between micronutrient and antioxidant intake and asthma. Therefore, our study agrees with the study conducted on adults that suggests dietary intake of antioxidants

and their main food sources have no effect on asthma risk (Nagel & Linseisen 2005) and contradicts those that propose protective roles for antioxidants against asthma (de Luis et al. 2005; Hatch 1995; McKeever & Britton 2004; Soutar, Seaton & Brown 1997).

4.3.2 Fatty acids

The mean level of total n-3 fatty acid intake was 1.31 g/day higher in those with no asthma compared with those who had asthma at both ages of eight and 14 ($p=0.009$), and those who had asthma at the age of 14 ($p=0.04$). Hence, it may be possible that the reduced levels of n-3 fatty acids have resulted in sustaining asthma in the latter group a result that is consistent with the literature. (Haby et al. 2001; Mickleborough et al. 2006; Oddy et al. 2004b; Peat et al. 2004) although there are some studies which do not find evidence that n-3 fatty acids are associated with a reduced risk of asthma (Almqvist et al. 2007; Woods et al. 2004).

N-3 fatty acids are suggested have a role in decreasing the production of inflammatory cytokines. It is suggested that these fatty acids act both directly (inhibiting arachidonic acid metabolism) and indirectly (altering the expression of inflammatory genes through effects on transcription factor activation) (Calder 2006). Our findings are consistent with these proposed roles of total n-3 fatty acids. We found that the lowest intake of n-3 fatty acids was observed in those individuals who had asthmatic symptoms at both ages of eight and 14 compared to the intake of n-3 fatty acids in non-asthmatics.

The mean intake of total n-6 fatty acids was 5.29 g/day higher in those without asthma history compared with the cases who did not have asthma at the age of eight but developed asthma later at the age of 14 ($p=0.009$). The total n-6 value was also 5.106 g/day higher for those who had asthma only at the age of eight compared to those who developed asthma at the age of 14 ($p=0.013$). The group who had asthma at both ages of eight and 14 had 5.340 g/day higher level of total n-6 fatty acids compared with those who had asthma only at the age of 14 ($p=0.011$). Only the latter

is in accordance with the proposed detrimental roles of n-6 fatty acids in asthma (McKeever 2007; Ogwok, Muyonga & Sserunjogi 2008).

An adverse effect of n-6 fatty acids is suggested in asthma (McKeever 2007), however, our study does not decisively support this. Arachidonic acid (20:4n6) is proposed to have a pro-inflammatory role as it is known to act as a substrate for 5-Lipoxygenase (5-LO) (Radmark et al. 2007). Therefore it was expected to observe a higher intake of this fatty acid in asthmatic individuals compared to non-asthmatics. However, our results showed that intake of arachidonic acid was significantly lower in asthmatic adolescents at 14 years compared to non-asthmatics (10.75 g/day vs. 13.41 g/day). It must be acknowledged that the number of adolescents in the group who had asthma at 14 years only, was very small (n=6 for group C) compared to other groups and consequently the mean intake of this fatty acid for this group may not represent an accurate estimate of the intake of arachidonic acid.

The lowest intake of n-6 fatty acids was observed in adolescents who had asthmatic symptoms at 14 years only. The intake of n-6 fatty acids was significantly higher for this group compared to non-asthmatic, asthmatics at eight years and asthmatic at both ages.

The level of n6:n3 also showed significant effects on the history of asthma. The mean ratio of n6:n3 was 0.592 higher in those who had asthma at both ages of eight and 14 years compared with those who never had asthma ($p=0.013$). This ratio was also 0.595 higher in the same group (those who had asthma at both ages) compared with the group who only had asthma at the age of eight ($p=0.025$). The group that had asthma at both ages also had 1.24 g/day higher ratio of n-6 to n-3 compared with the group who did not have asthma at the age of eight but did develop asthma at the age of 14 ($p=0.038$).

The ratio of n6:n3 was observed to be highest for the asthmatic individuals at both ages of eight and 14. This ratio was significantly higher compared to non-asthmatic, asthmatic at eight years and asthmatic at 14 years only. These findings are consistent with the literature as significant evidence was previously found in this cohort for a modulatory effect of the dietary n-6:n-3 fatty acid ratio on the presence of asthma in

children (Oddy et al. 2004a). Linoleic acid (18:2n6) and α -linolenic (18:3n3) and their long-chain derivatives are essential components of cell membranes both in animals and plants (Simopoulos 2002). These two classes of fatty acids are metabolically and functionally separate, often have important opposing physiological functions and are not interchangeable (Riediger et al. 2009; Simopoulos 2002). While cellular proteins are genetically programmed, the fatty acid composition of the cell membranes is greatly dependent on dietary intake (Leemans et al. 2009). Linoleic acid and α -linolenic are known to be the precursor for eicosanoid (prostaglandins, thromboxane and leukotrienes) production (Riediger et al. 2009). It is proposed that due to the increased amounts of n-6 fatty acids in the Western diet, the eicosanoid metabolic products are formed in larger quantities than those formed from n-3 fatty acids. The eicosanoids derived from n-6 fatty acids are biologically very active even in small quantities and therefore, when they are formed in large amounts; they could contribute to the formation of allergic and inflammatory disorders (Mickleborough et al. 2009; Simopoulos 2002).

After adjusting for confounders, the association between the intakes of arachidonic acid, total n-3 and n-6 and asthma were shown as non-significant. However, the association between the level of n6:n3 in non-asthmatics (2.78) and those with asthma at eight and 14 years (3.38) remained significant ($p=0.030$).

4.4 COMPARISON OF THE RESULTS OF TWO STUDIES

In Study One a protective association between higher consumption of vitamin C and asthma was observed. In the same study, a protective association between higher intake of berry fruits with atopy and wheezing episodes was also found, however, in Study Two no significant associations between asthmatic symptoms and consumption of these fruits and nutrients were observed.

The analysis of fatty acid intake in Study One used red blood cell fatty acid content, while in Study Two fatty acid intake was estimated from the food consumed during the day as they were recorded in food frequency questionnaires. Therefore, the information on fatty acids in Study One may be more applicable as they are objective

measurements rather than relying on memories of the participants or their parents. However, the food frequency questionnaires used in both studies were based on the Commonwealth Scientific and Industrial Research. These questionnaires were validated in adults (Yearsley, Last & Ward 1999) and had previously been applied in children (Oddy et al. 2004b). Therefore, the outcome data measurements were based on validated methods and questionnaires.

In Study One, we found a number of significant associations between the level of individual fatty acids in red blood cells and asthmatic outcomes. In crude analysis docosapentaenoic acid (22:5n3) in red blood cells was positively associated with asthma, BHR and wheezing episodes. However, after adjusting for confounders (age, sex, BMI and total energy intake), no significant associations between the level of docosapentaenoic acid and any of the asthmatic symptoms was found.

In Study One a significant protective association between the adjusted level of docosahexaenoic acid (DHA) (22:6n3) and BHR was shown.

In both studies the ratio of n6:n3 fatty acids was demonstrated to have a significant detrimental association with asthmatic symptoms.

Besides having two different methods for collecting data on fatty acids levels in these two studies as discussed, we also had a larger number of red blood cell samples (n=1329) in Study One compared to the number of food frequency questionnaires (n=335) in Study Two. Therefore, it could be argued that there was a better probability to discover associations between fatty acids and asthma in Study One as opposed to Study Two. However, we could look for the differences longitudinally in Study Two and explore any possible differences in the cohort over the period of six years.

4.5 STRENGTH AND LIMITATIONS OF THE TWO STUDIES

Food frequency questionnaires were used for collection of dietary intake data in both studies. The use of food frequency questionnaires has become the primary method for measuring dietary intake in epidemiological studies (McNamara, Sullivan & Richtand 2008; Willett 1998). The main advantages of using food frequency questionnaires were that they are easy for the subjects to complete and their processing can readily be computerised. The food frequency questionnaire in our study was validated in adults (Yearsley, Last & Ward 1999) and has earlier been validated and applied for fish intake against serum fatty acids samples in the eight year follow-up in children in the Raine study (Oddy et al. 2004b).

One of the limitations of this study was that it relied on the use of participants' long-term memories to record usual intake of a wide range of foods. Therefore, the existence of recall bias cannot be excluded as it has been documented in other epidemiological studies relying on the participants long-term memories (Broadfield et al. 2004; Huang, Lin & Pan 2001).

The presence of the temptation to exaggerate intake of foods perceived as healthy by the participants and under-reporting of food that may be perceived as unhealthy was another limitation. Nutrients may synergistically provide comprehensive modulatory inflammatory reactions (Yeum et al. 2009). Therefore, any effects of diet observed on asthma may have come from combined interactions of foods in diet and not the result of a single nutrient or food.

The Western Australian Pregnancy Cohort Study had a large number of participants, and thus provided sufficient power to detect statistically significant differences between adolescents with and without asthma. There was only one group in Study Two (asthma at 14 years) that contained a very small number of participants (n=6). The small number of the participants in this group resulted in a large variation in the intake of foods and nutrients as demonstrated by a large Standard Error of Mean (SEM) in this group. Therefore, the intake of foods in this group may not represent a

precise estimate of the intake of these nutrients and hence the differences observed between this group and the others may not be reliable.

4.6 *DIETARY RECOMMENDATIONS*

Based on our findings it is recommended to include more berry fruits in the diets of children and adolescents. We also suggest including more fruits containing high levels of vitamin C in the diets. It is also recommended to reduce the ratio of n6:n3 fatty acids by including more foods which are high in n-3 fatty acids (whole grains, fatty fish, flax seeds and soy bean) and reducing foods that are high in n-6 fatty acids (poppy seed oil, palm oil, sunflower oil, soybean oil, coconut oil, peanut oil, wheat germ oil, grape seed oil, macadamia oil, pistachio oil and sesame oil). The most widely available dietary source of EPA and DHA is cold water oily fish, such as salmon, herring, mackerel, anchovies and sardines. Oils from these fish have a profile of around seven times as much n-3 as n-6 (Antonijevic et al. 2007). It is however advisable that a dietician be consulted to address individual requirements for dietary aspects of increasing n-3 fatty acids.

CHAPTER FIVE

5.1 CONCLUSIONS

The objective of this thesis was to gain insight into possible associations between the intake of dietary antioxidants, n-3 and n-6 fatty acids as well as red blood cell level of these fatty acids with asthmatic traits in children and adolescents.

In the following sections, I provide suggestions for future research and the final summary of this study.

5.2 FUTURE STUDIES

There are inconsistencies in the evidence of the roles of different nutrients on asthma in different populations (Almqvist et al. 2007; Emecen et al. 2009; Leemans et al. 2009; McKeever 2007; McNamara, Sullivan & Richtand 2008; Mickleborough, Ionescu & Rundell 2004; Oddy et al. 2004a; Riediger et al. 2009; Schmitz & Ecker 2008; Takaoka & Norback 2008). One likely reason for this irregularity in the evidence is that assortments of different methods both in biomedical and epidemiological investigations have been used to measure nutrients and fatty acids. Therefore, future studies should concentrate on providing standard methods in measurements of biological and population variables (such as the validated FFQ that was used in this study). These methods could be applied in a uniform format across different populations worldwide.

Larger epidemiological cohort studies may be required to ascertain the mechanisms implicated by fatty acids in immune system regulation throughout the life course, and to establish a better understanding of the association between total fatty acid intake and respiratory health.

More investigations are necessary to search for differences in the diets of children in countries with lower incidence of asthma compared with Western countries aiming to

establish the possible effects of other nutrients which could have an impact in reducing the risk of asthma and atopic disease in those countries.

The changes in lifestyle that have occurred in developed countries compared to developing countries have not been limited to the changes in diets and other factors, such as differences in the level of physical activity. These differences may have contributed to increases in the prevalence of asthma and other allergic diseases compared with developing countries. Despite the fact that there is a recent study which rules out the role of physical activity (Castro-Rodriguez et al. 2008), more evidence should be gathered. This can be achieved by studying details of physical activity in children and adolescents in a large scale study and exploring possible associations between asthma and different level of these activities as well as nutrition.

5.3 FINAL SUMMARY

This research was conducted to investigate the hypothesis that consumption of antioxidants nutrients, n-3 fatty acids, fresh fruits and vegetables with a high content level of antioxidants will reduce asthmatic symptoms in adolescents. This study was relied on the use of participants' long-term memories to record the intake of a wide range of foods. The existence of recall bias cannot be excluded in this study.

We found some evidence that a decreased in consumption of some fresh fruits (berry fruits) may be associated with asthmatic symptoms.

Most of the dietary antioxidants did not show a protective effect on asthma in 14 year old adolescents. This may be due to the fact that there are autoimmune components involved in the manifestation of asthma that cannot be modulated by dietary intake of fruits and vegetables. We found that by increasing intake of vitamin C we may reduce the risk of developing asthma. Higher level of docosahexaenoic acid in RBC membrane was associated with a reduced risk of BHR.

We also demonstrated that an increase in the ratio of n6:n3 was associated with more asthmatic symptoms. The adjusted ratio of n6:n3 showed a detrimental association with asthma and wheezing episodes. We propose that the ratio of n6:n3 in diet may play a modulatory role in the expression of asthma, suggesting that promotion of increased total n-3 and reduced n-6 in the diet may protect against asthmatic traits in childhood and early adolescence. Further studies are required to confirm these findings given the limitation of this study.

There are numerous questions remaining related to the inherent complexities concerning the role of diet in modulation of asthmatic symptoms. It remains unclear to what extent ALA is converted to EPA and DHA in human body.

In Study Two the number of adolescents in group C (asthma at 14 years only) was very low (n=6) and therefore, the results concerning this group have to be interpreted with caution due to the low statistical power (11.4%) of the study.

In conclusion, there are still many uncertainties and inconsistencies on the role of diet on asthma and allergies. One of the major reasons that studies into the diet and its role in the development of allergies remains so inconclusive may be the complexity of the interaction between nutrients and the immune system.

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Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.

Appendix 1 – Approved Ethics Certificate


| MINUTE | | Curtin <small>UNIVERSITY OF TECHNOLOGY</small> |
|----------------|--|---|
| To | Mr Ramin Nikravan | SCHOOL OF PUBLIC HEALTH TELEPHONE 9266 4346 FACSIMILE 9266 2958 EMAIL l.thompson@curtin.edu.au |
| From | Leslie Thompson | |
| Subject | Protocol Approval – SPH – 0019 - 2007 | |
| Date | June 12 th , 2007 | |
| Copy | Dr Wendy Oddy | |

Dear Ramin

Thank you for your "Form C Application for Approval of Research with Minimal Risk (Ethical Requirements)" for the project titled "A Western Australian cohort study investigation on the role of antioxidants in asthmatic traits in children". On behalf of the Human Research Ethics Committee I am authorised to inform you that the project has been approved.

Approval of this project is for a period from June 12th, 2007 to December 31st, 2008.




If at any time during the twelve months changes/amendments occur, or if a serious or unexpected adverse event occurs, please advise me immediately. The approval number for your project is SPH – 0019 - 2007. *Please quote this number in any future correspondence.*



Leslie Thompson
Coordinator
Human Research Ethics Form C
School of Public Health

Please Note: The following standard statement must be included in the information sheet to participants:
This study has been approved by the Curtin University Human Research Ethics Committee. If needed, verification of approval can be obtained either by writing to the Curtin University Human Research Ethics Committee, c/- Office of Research and Development, Curtin University of Technology, GPO Box U1987, Perth, 6845 or by telephoning 9266 2784.

Appendix 2 – Year 13 Ethics Approval

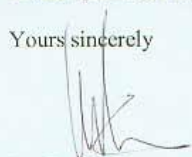
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|--|--|---|
|  | Department of Health Government of Western Australia |  |
| <p>Professor Peter Sly Division of Clinical Sciences Institute for Child Health Research SUBIACO WA 6008</p> | | |
| <p>Dear Professor Sly</p> | | |
| REGISTRATION NUMBER: | 846/EP | |
| TITLE: | The Raine study – physical activity levels, respiratory disease and hypothalamo-pituitary-adrenal responsiveness in early adolescence | |
| REFERENCE NUMBER: | EC03-14.7 | |
| MEETING DATE: | 20 March 2003 | |
| <p>The Ethics Committee has recommended approval be given for you to undertake the abovenamed research study. This recommendation has been ratified by the Women's and Children's Health Service.</p> | | |
| <p>The Ethics Committee does however wish to be informed immediately of:</p> | | |
| <p>I. any untoward effects experienced by any participant in the trial where those effects in degree or nature were not anticipated by the researchers, and steps taken to deal with these,</p> | | |
| <p>II. substantial changes in the research protocol together with an indication of ethical implications, and</p> | | |
| <p>III. other unforeseen events.</p> | | |
| <p>The Ethics Committee has been charged with the responsibility of keeping the progress of all approved research under surveillance. A copy of the final result must be forwarded to the Committee upon completion of the research or if the research is not completed within twelve months you are asked to submit a progress report and annually thereafter. This information should include:</p> | | |
| <p>a) The status of the project (completed/in progress/abandoned/not commenced). In the event that a project does not commence within 12 months of being approved by the Ethics Committee the study must be resubmitted to the Committee for approval.</p> | | |
| <p>b) Compliance with conditions of ethical approval, including security of records and procedures for consent.</p> | | |
|  | | |
| <p>King Edward Memorial Hospital for Women 374 Beagar Road Subiaco WA 6008 PO Box 134 Subiaco WA 6904 Tel: (08) 9340 2222 Fax: (08) 9368 1780</p> | | |
| <p>Princess Margaret Hospital for Children Roberts Road Subiaco WA 6008 GPO Box D184 Perth WA 6840 Tel: (08) 9340 8222 Fax: (08) 9340 6111</p> | | |

- c) Compliance with any special conditions stated by the Ethics Committee as a condition of approval.
- d) Results from the study to date, including outcome.

Please note that approval for studies is for three years and the research should be commenced and completed within that period of time. Projects must be resubmitted if an extension of time is required. In the event that a project does not commence within 12 months of being approved by the Ethics Committee the study must be resubmitted to the Committee for approval.

Please quote the above registration number on all correspondence.

Yours sincerely



**Dr Geoff Masters
Executive Director
Medical Services**

27 March 2003

cc Raine Study Co-ordinator

- **The Ethics Committee is constituted, and operates in accordance with the National Health and Medical Research Council's National Statement on Ethical Conduct in Research Involving Humans**

Appendix 3 – Year 13 Primary Parent

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TELETHON INSTITUTE FOR CHILD HEALTH RESEARCH

WESTERN AUSTRALIAN
PREGNANCY COHORT (RAINE) STUDY

13 YEAR FOLLOW UP QUESTIONNAIRE

Primary Caregiver

❖ **Thank you for continuing to help us with the Raine Study.**

The purpose of this questionnaire is to obtain information about your child's home life, leisure activities, schooling, behaviour and general health since we were last in contact. The questionnaire is similar to those you have completed in the past, but there are additional questions about your health and happiness and level of physical activity

❖ **Please read each question carefully.**

Write your answers in the space provided or circle the most appropriate option.

❖ **Please take your time.**

If you are uncomfortable about a question or unsure of an answer, please leave it blank and discuss it with one of the Raine Study staff when you come in, or phone us at 9489 7794, 9489 7793 or 9489 7796.

❖ **Remember all answers are STRICTLY confidential.**

❖ **Please complete this questionnaire as soon as possible.**

If you are coming in for an appointment, please bring your completed questionnaire with you on the day. If you are unable to attend, please return the questionnaire in the Reply Paid envelope provided by: _____

Office use only

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Section 1

Here are some questions for you that are similar to ones we have asked in previous years.
We are keen to know if any of these things have changed *since you were last asked*.

Please write the answer in the space provided or circle the answer where applicable.

HOUSING AND FAMILY - STRICTLY CONFIDENTIAL

- Q1. How old is your house/flat (approximately)? _____ years

| | |
|--|--|
| | |
|--|--|
- Q2. How many bedrooms are there? _____

| |
|--|
| |
|--|
- Q3. How many bathrooms are there? _____

| |
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| |
|--|
- Q4. Have you moved house since the last time you completed a Raine Study questionnaire? (i.e. in the last three years)

| |
|--|
| |
|--|

0 No

1 Yes How many times?

| | |
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| | |
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Q5a. If you live in Australia, what is your current residential postcode? _____

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

Q5b. If you live overseas, please indicate which country _____

| | | |
|--|--|--|
| | | |
|--|--|--|

Q6. How many adults and children live in your home?
(Please include your study child and yourself)

| First name | Age yrs | Sex M/F | Relationship to study child |
|---------------|---------|---------|-----------------------------|
| eg. Elizabeth | 42 | F | mother |
| eg. David | 35 | M | stepfather |
| eg. Jessica | 13 | F | study child |
| eg. Hannah | 2 | F | stepsister |

| | | | | | | | | |
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Office use only

Q7. Does your child have any other brothers or sisters not mentioned in Q6?

0 No Go to Q8

1 Yes

☐

| First name | Age yrs | Sex M/F | Relationship to study child |
|------------|---------|---------|-----------------------------|
| eg. Rachel | 18 | F | sister |
| eg. Simon | 22 | M | stepbrother |
| e.g. Tom | 3 | M | half brother |
| | | | |
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Q8. Is the father (mother) of the study child (your 13 year old) living with you?

2 Yes Go to Q12

1 Not applicable – father (mother) deceased Go to Q11

0 No Go to Q9

☐

| | |
|--|-----|
| Q9. Do you have any social contact with him/her? | |
| 0 | No |
| 1 | Yes |
| Q10. Does he/she provide any financial support for the care of your child? | |
| 0 | No |
| 1 | Yes |
| Q11. Do you have another partner who lives with you? | |
| 0 | No |
| 1 | Yes |

☐☐☐

Office use only

Q12. Are you or your partner receiving a benefit?☐

0 No Go to Q14

1 Yes



Q13. Which benefit(s) are you or your partner receiving?

(Please circle *all* appropriate answers)

- 0 Sole parent's benefit
- 1 Unemployment benefit
- 2 Disability allowance – parent
- 3 Disability allowance – child
- 4 Workers compensation
- 5 Sickness benefit
- 6 Austudy/Abstudy
- 7 Other Please specify

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Q14. Do you currently have a full-time or part-time job of any kind (excluding home duties)?
(Please circle *one* answer only – the main job)☐

- 0 No, do not have a job – not seeking work Go to Q18
- 1 No, do not have a job – actively seeking work Go to Q18
- 2 Yes, work for payment or profit
- 3 Yes, unpaid work in a family business } Go to Q15
- 4 Yes, other unpaid work }



Q15. In your main job (if you have more than one job, then 'main job' refers to the job in which you usually work the most hours) are you:

(Please circle *one* answer only)

- 0 A salary or wage earner
- 1 A helper not receiving wages
- 2 Conducting your own business – with employees
- 3 Conducting your own business – without employees

☐

Q16. Describe your current main job. (Please give title of job and description of work in detail)

Job

Description

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Q17. How many hours do you usually work in all jobs?
☐
☐
☐

- 0 None or less than one hour
- 1 One or more hours per week (Please specify)

Office use only

Q18. What do you currently spend most of your time doing? *(Please only circle one answer, unless two, or more, answers apply equally)*

- 0 Full-time or part-time job (salary or own business)
- 1 Voluntary work
- 2 Looking for work
- 3 Home duties / caring for children
- 4 Studying
- 5 Voluntarily out of the workforce / retired
- 6 Recovering from injury / illness
- 7 Caring for an aged / disabled / ill person
- 8 Other (Please describe)

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Q19. Does your partner currently have a full-time or part-time job of any kind (excluding home duties)? *(Please circle one answer only – the main job)*

☐

- 0 No partner
- 1 No, does not have a job – not seeking work
- 2 No, does not have a job – actively seeking work
- 3 Yes, works for payment or profit
- 4 Yes, unpaid work in a family business
- 5 Yes, other unpaid work

Go to Q24
Go to Q23
Go to Q23



Q20. In your partner's main job (if he/she has more than one job, then 'main job' refers to the job in which he/she usually works the most hours) is he/she: *(Please circle one answer only)*

☐

- 0 A salary or wage earner
- 1 A helper not receiving wages
- 2 Conducting his/her own business – with employees
- 3 Conducting his/her own business – without employees

Q21. Describe your partner's current main job. *(Please give title of job and description of work in detail)*

Job
Description
.....

☐

Q22. How many hours does your partner usually work in all jobs?

☐

- 0 None or less than one hour
- 1 One or more hours per week *(Please specify)*

Q23. What does your partner currently spend most of his/her time doing? *(Please circle one answer unless two, or more, answers apply equally)*

- 0 Full-time or part-time job (salary or own business)
- 1 Voluntary work
- 2 Looking for work
- 3 Home duties / caring for children
- 4 Studying
- 5 Voluntarily out of the workforce / retired
- 6 Recovering from injury / illness
- 7 Caring for an aged / disabled / ill person

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8 Other (Please describe)

Office use only

Q24. What is your total family income (before tax) per year now? (Please include income from investments, rent assistance, maintenance, family supplement, etc)

| | | |
|----|--------------------------------|-------------------------------|
| 0 | \$1 to \$8,000 per year | (\$1 to \$154 per week) |
| 1 | \$8,001 to \$16,000 per year | (\$155 to \$308 per week) |
| 2 | \$16,001 to \$25,000 per year | (\$309 to \$481 per week) |
| 3 | \$25,001 to \$30,000 per year | (\$482 to \$577 per week) |
| 4 | \$30,001 to \$35,000 per year | (\$578 to \$673 per week) |
| 5 | \$35,001 to \$40,000 per year | (\$674 to \$769 per week) |
| 6 | \$40,001 to \$50,000 per year | (\$770 to \$962 per week) |
| 7 | \$50,001 to \$60,000 per year | (\$963 to \$1,154 per week) |
| 8 | \$60,001 to \$70,000 per year | (\$1,155 to \$1,346 per week) |
| 9 | \$70,001 to \$78,000 per year | (\$1,347 to \$1500 per week) |
| 10 | \$78,001 to \$104,000 per year | (\$1,501 to 2,000 per week) |
| 11 | \$104,000 or more per year | (>\$2,000 per week) |

How many people does this income support?:

Adults and children over 14 yrs: _____ Children: _____

Q25. What best describes your situation with regard to the house, unit, flat or other residence you live in? (Please circle **one** answer only)

- 1 Being paid off by you (or your spouse/partner)
- 2 Owned outright by you (or your spouse/partner)
- 3 Rented by you (or your partner)
- 4 Being purchased under a rent/buy (or shared equity) scheme by you (or your spouse/partner)
- 5 Occupied under a life tenure scheme
- 6 None of these
- 7 Don't know

The next two questions are about the neighbourhood in which **you** live.

Q26. To what extent do you agree or disagree with these statements about your neighbourhood?

| Know | | Strongly Agree | Agree | Disagree | Strongly Disagree | Don't Disagree |
|------|---|----------------|-------|----------|-------------------|----------------|
| 1 | This is a safe neighbourhood | 4 | 3 | 2 | 1 | 0 |
| 2. | This is a clean neighbourhood | 4 | 3 | 2 | 1 | 0 |
| 3. | There are good parks, playgrounds and play spaces in this neighbourhood | 4 | 3 | 2 | 1 | 0 |
| 4. | There is good street lighting in this neighbourhood | 4 | 3 | 2 | 1 | 0 |
| 5. | The state of the footpaths and roads is good in this neighbourhood | 4 | 3 | 2 | 1 | 0 |

Office use only

Q27. Over the last two years, have any of the following been a problem in your neighbourhood? (Please circle **one** answer for each item)

| | Yes | No | Don't Know |
|-----------------------------------|-----|----|------------|
| a. Vandalism or graffiti | 2 | 1 | 0 |
| b. House burglaries | 2 | 1 | 0 |
| c. Car theft or damage | 2 | 1 | 0 |
| d. Domestic violence | 2 | 1 | 0 |
| e. Violence in the streets | 2 | 1 | 0 |
| f. Drug or alcohol abuse | 2 | 1 | 0 |
| g. Noisy or reckless driving | 2 | 1 | 0 |
| h. Racist discrimination or abuse | 2 | 1 | 0 |

YOUR HEALTH AND WELLBEING – STRICTLY CONFIDENTIAL

The following questions ask about the health and wellbeing of the study child's biological mother and father. We are also interested to know about the health and wellbeing of your partner if the father (mother) of your child is no longer living with you. We have tried to keep these to a minimum but some things that affect parents may also affect their children.

Q28. Do you smoke cigarettes?

0 No Go to Q32

1 Yes



Q29. How many cigarettes do you smoke a day now?

- 0 Less than 1 daily
- 1 1-5 daily
- 2 6-10 daily
- 3 11-15 daily
- 4 16-20 daily
- 5 More than 20 daily

Q30. Do you smoke inside your house?

- 0 No
- 1 Yes

Q31. Do you smoke in the car?

- 0 No
- 1 Yes

☐
☐
☐
☐

Office use only

Q32. Does anyone else living in your house smoke cigarettes?

0 No Go to Q36

1 Yes



Q33. How many do they smoke a day now? (If more than one person at home smokes, please circle the total number of cigarettes smoked)

- 0 Less than 1 daily
 1 1-5 daily
 2 6-10 daily
 3 11-15 daily
 4 16-20 daily
 5 More than 20 daily

Q34. Do they smoke inside your house?

0 No

1 Yes

Q35. Do they smoke in the car?

0 No

1 Yes

Q36. Does anyone at your home smoke/use any other substances? (Please include pipe, cigars, marijuana, other drugs, etc)

0 No

1 Yes - once a week or less

2 Yes - more than once weekly but not every day

3 Yes - every day

What do they smoke/use?

Q37. In general how would you describe your health?

| | Mother | Father | Partner |
|-----------|--------|--------|---------|
| Poor | 0 | 0 | 0 |
| Fair | 1 | 1 | 1 |
| Good | 2 | 2 | 2 |
| Very Good | 3 | 3 | 3 |
| Excellent | 4 | 4 | 4 |

Q38. Do you have any medical conditions or health problems of a permanent or long term nature (that is, for more than 6 months)?

| | | |
|---------|----|-----|
| Mother | No | Yes |
| Father | No | Yes |
| Partner | No | Yes |

Office use only

Q39. Are you limited in any way in carrying out normal daily activities at home, at a job or in studying, because of a medical condition or health problem?

☐ ☐ ☐

| | | |
|----------------|----|-----|
| Mother | No | Yes |
| Father | No | Yes |
| Partner | No | Yes |

Q40. Has the study child's mother ever had post-natal depression? (*Please circle all appropriate answers*)

| | |
|---|--|
| 0 | No |
| 1 | Yes, with a child(ren) born before the study child |
| 2 | Yes, with a child(ren) born after the study child |
| 3 | Yes, associated with the birth of the study child |
| 4 | Don't know, unsure |

☐
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Q41. Have you ever been treated for an emotional or mental health problem (other than post-natal depression)?

☐ ☐ ☐

| | | |
|----------------|----|-----|
| Mother | No | Yes |
| Father | No | Yes |
| Partner | No | Yes |

Q42. Have you been treated for an emotional or mental health problem within the last 6 months?

☐ ☐ ☐

| | | | |
|----------------|----|-----|---------------------------|
| Mother | No | Yes | N/A (never had treatment) |
| Father | No | Yes | N/A (never had treatment) |
| Partner | No | Yes | N/A (never had treatment) |

Q43. Have you ever been hospitalised for an emotional or mental health problem?

☐ ☐ ☐

| | | | |
|----------------|----|-----|---------------------------|
| Mother | No | Yes | N/A (never had treatment) |
| Father | No | Yes | N/A (never had treatment) |
| Partner | No | Yes | N/A (never had treatment) |

Q44a. On average, over the past 6 months, about how many drinks of beer, wine, spirits or other alcoholic beverage have you taken.

| | Mother | Father | Partner | |
|---------------------------|--------|--------|---------|-------------|
| Don't drink alcohol | 0 | 0 | 0 | Go to Q. 45 |
| Less than 3 drinks a week | 1 | 1 | 1 | |
| 3 - 6 drinks a week | 2 | 2 | 2 | |
| 1 or 2 drinks a day | 3 | 3 | 3 | |
| 3 - 6 drinks a day | 4 | 4 | 4 | |
| More than 6 drinks a day | 5 | 5 | 5 | |

Q44b. Please indicate, as accurately as possible, the type and amount of alcohol consumed each day during the past week.

Type of alcohol: Examples: Beer (please specify brand and strength)
Wine (Sherry, Claret, Chardonnay, etc)
Spirits (Gin, Whiskey, Baileys, etc)

Amount Consumed: Indicate the number of glasses, cans, stubbies, nips, or mls (if you know it) etc...Whatever measures you are most familiar with.

Start with yesterdays drinks and work back through the whole week. If you didn't have anything to drink on a particular day, please write NIL in the "Amount Consumed" column.

| DAY | TYPE OF ALCOHOL | AMOUNT CONSUMED | |
|-----------|-----------------|-----------------|----------------------|
| Monday | | | <input type="text"/> |
| Tuesday | | | <input type="text"/> |
| Wednesday | | | <input type="text"/> |
| Thursday | | | <input type="text"/> |
| Friday | | | <input type="text"/> |
| Saturday | | | <input type="text"/> |
| Sunday | | | <input type="text"/> |

Q44c. Does this level of consumption reflect a typical week?

0 No
1 Yes

☐

Q45. Have you ever had back pain?

0 No Go to Q50

1 Yes

☐

Q46. Did you seek health professional advice/treatment?

0 No

1 Yes

☐

Q47. Did you take medication to relieve the pain?

0 No

1 Yes

☐

Q48. Did you miss work due to the pain?

0 No

1 Yes

☐

Q49. Did the pain interfere with your normal activities?

0 No

1 Yes

☐

Section 2

The next few questions (Q50-52a) are about the physical activity you did last week, outside of that which results from your work.

Q50. In the last week how many times have you walked continuously, for at least 10 minutes, for recreation/exercise, or to get to and from places?

The next question excludes household chores, gardening or yard work.

Q51. In the last week, how many times did you do any moderate/vigorous physical activity which made you breathe harder or puff and pant? (e.g. jogging, cycling, aerobics, competitive tennis)

The next question includes household chores, gardening or yard work.

Q52a. In the last week how many times did you do any moderate/vigorous household chores, gardening or heavy work around the yard which made you breathe harder or puff and pant?

Q52b. Does the level of activity detailed in Questions 50-52a reflect a typical week?

0 No

1 Yes

☐

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Q53. Do you belong to: *(Please circle all appropriate answers)*

| | | |
|-------------------------------------|----|-----|
| A sports club | No | Yes |
| An exercise club | No | Yes |
| An outdoor recreation club or group | No | Yes |

☐
☐
☐

Q54. What is the MAIN reason (s) for you doing physical activity?
(Please circle all appropriate answers)

- 0 Improve appearance
- 1 Enjoy doing the activity
- 2 Maintain or lose weight
- 3 Social interaction and friendships
- 4 Reduce my risk of heart disease
- 5 Feel more relaxed
- 6 Tone my muscles
- 7 Improve my fitness
- 8 Feel better about my self
- 9 Have more energy
- 10 Sleep better
- 11 Prevent joint stiffness
- 12 Other
- 13 No reason

☐
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Q55. Who normally does physical activity with you?
(Please circle all appropriate answers)

- 1 Spouse/partner
- 2 The child in the study
- 3 Another of your children
- 4 Friend
- 5 Workmate
- 6 Neighbour
- 7 Sports or health club member
- 8 No-one
- 9 Children other than your own (coaching)
- 10 Pets
- 11 Other

☐
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Office use only

The following statements are about the amount of exercise you intend to do in the near future.

Q56. Do you intend to be more active than you have been over the last week?

☐

- 0 No
- 1 Yes
- 2 Unsure

Q57. What reasons would you give for not being more physically active? (*Please circle all appropriate answers*)

- 0 I haven't got time ☐
- 1 My health is not good enough ☐
- 2 There is no one to do it with ☐
- 3 I've lost contact with friends/family ☐
- 4 I can't afford it ☐
- 5 I'm too old ☐
- 6 There are no suitable facilities ☐
- 7 Traffic is too heavy ☐
- 8 I'm not the sporty type ☐
- 9 No motivation ☐
- 10 Can't be bothered ☐
- 11 Too fat – overweight ☐
- 12 I need to rest and relax in my spare time ☐
- 13 I don't put priority on physical activity ☐
- 14 I've got young children to look after ☐
- 15 I might get injured or damage my health ☐
- 16 I don't enjoy physical activity ☐
- 17 I'm active enough ☐
- 18 Other (specify) ☐
- 19 No reason ☐

To what extent do you agree or disagree with the following statement about physical activities?

Q58. Taking the stairs at work or generally being more active for at least 30 minutes each day is enough to improve your health.

☐

- 0 Agree
- 1 Neither agree nor disagree
- 2 Disagree

Q59. Half an hour of brisk walking on most days is enough to improve your health.

☐

- 0 Agree
- 1 Neither agree nor disagree
- 2 Disagree

Q60. To improve your health it is essential for you to do vigorous exercise for at least 20 minutes each time, 3 times per week.

☐

- 0 Agree
- 1 Neither agree nor disagree
- 2 Disagree

Q61. Exercise doesn't have to be done all at one time – blocks of 10 minutes are okay.

☐

- 0 Agree
- 1 Neither agree nor disagree
- 2 Disagree

Q62. Moderate exercise that increases your heart rate slightly can improve your health.

☐

- 0 Agree
- 1 Neither agree nor disagree
- 2 Disagree

Q63a. On average how many hours per day do you spend watching television or videos?

☐

- 0 None at all
- 1 Up to one hour a day
- 2 1-2 hours a day
- 3 2-3 hours a day
- 4 4 hours or more a day

Q63b. On average how many hours per day do you spend using a computer?

☐

- 0 None at all
- 1 Up to one hour a day
- 2 1-2 hours a day
- 3 2-3 hours a day
- 4 4 hours or more a day

Please select the most appropriate response for the following questions

- Q64. Do you know your weight?
 0 No Go to Q66 ☐
 1 Yes
- Q65. What is your current weight?
kg orstone
- Q66. Are you worried about your weight?
 0 Not at all ☐
 1 A little
 2 Moderately
 3 Very
- Q67. Do you consider yourself to be? ☐
 0 Underweight
 1 Normal weight
 2 A bit overweight
 3 Very overweight
- Q68. Are you worried about your child's weight? ☐
 0 Not at all
 1 A little
 2 Moderately
 3 Very
- Q69. Do you consider your child to be? ☐
 0 Underweight
 1 Normal weight
 2 A bit overweight
 3 Very overweight
- Q70. How much does your weight and shape influence how you think about (judge) yourself? ☐
 0 Not at all
 1 A little
 2 Moderately
 3 Very
- Q71. How much does your weight and shape influence how you think about (judge) others? ☐
 0 Not at all
 1 A little
 2 Moderately
 3 Very

Please select one number only for each question. Circle the number which applies to your diet.

Q72. How often do you eat the following foods?

| | 6 + times a week | 3-5 times a week | 1-2 times a week | 1-2 times a month | Rarely or never |
|--|------------------------|------------------------|------------------------|-------------------------|-----------------------|
| Fried food with a batter or breadcrumb coating | 4 | 3 | 2 | 1 | 0 |
| Gravy, creamy sauces or cheese sauces | 4 | 3 | 2 | 1 | 0 |
| Vegetables, rice or pasta with added butter, margarine, oil or sour cream | 4 | 3 | 2 | 1 | 0 |
| Vegetables that are fried or roasted with fat or oil (don't count oil sprays eg Pure and Simple) | 4 | 3 | 2 | 1 | 0 |
| Sausages, polony, salami, meat pies, pasties, hamburger or bacon | 4 | 3 | 2 | 1 | 0 |
| Hot potato chips or French fries | 4 | 3 | 2 | 1 | 0 |
| Pastries, cakes, sweet biscuits or croissants | 4 | 3 | 2 | 1 | 0 |
| Chocolate, chocolate biscuits or sweet snack bars | 4 | 3 | 2 | 1 | 0 |
| Potato crisps, corn chips, cheezels, twisties or nuts | 4 | 3 | 2 | 1 | 0 |
| Ice cream (any variety) | 4 | 3 | 2 | 1 | 0 |
| Cream or sour cream | 4 | 3 | 2 | 1 | 0 |
| Cheddar, edam or other hard cheese, cream cheese or soft cheeses such as camembert or brie (but excluding ricotta or cottage cheese) | 4 | 3 | 2 | 1 | 0 |

Q73. How much of the following do you usually eat?

| | Most or all | Some | None | Don't eat this food |
|-----------------|-------------|------|------|------------------------|
| Fat on meat | 3 | 2 | 1 | 0 |
| Skin on chicken | 3 | 2 | 1 | 0 |

Q74. How often do you eat the following foods?

| | 6 + times a week | 3-5 times a week | 1-2 times a week | 1-2 times a month | Rarely or never |
|---|------------------------|------------------------|------------------------|-------------------------|-----------------------|
| Fruit. including fresh and canned fruit (Do not include dried fruit, fruit juices, fruit drinks, fruit bars or frozen fruit deserts) | 4 | 3 | 2 | 1 | 0 |
| Vegetables. Include all forms of vegetables, e.g. fresh, frozen, canned and salads | 4 | 3 | 2 | 1 | 0 |

Q75. What type of milk do you usually use? (*Please circle one answer only*).

- 1 Condensed
- 2 Full – cream
- 3 Reduced fat (2%) e.g. hilo or reduced fat soy
- 4 Skim
- 5 None

☐

Q76. How much butter/margarine do you usually use on bread? (*Please circle one answer only*).

- 1 Thick spread
- 2 Medium spread
- 3 Thin spread
- 4 None

☐

Q77. For each one of the following foods you eat, circle the **most common** cooking method used.
(*Please circle one answer only for each item*)

| | Boiled, steamed or micro waved | Stewed or casseroled | Dry baked, dry fried or grilled | Baked, fried or roasted with fat/oil | Don't eat |
|----------------|---|-------------------------|---------------------------------------|--|-----------|
| Beef/lamb/pork | 4 | 3 | 2 | 1 | 0 |
| Sausages | 4 | 3 | 2 | 1 | 0 |
| Poultry | 4 | 3 | 2 | 1 | 0 |
| Fish | 4 | 3 | 2 | 1 | 0 |
| Vegetables | 4 | 3 | 2 | 1 | 0 |

Q78. From the following list, circle the fruits which you eat *at least once a week* (on average), when they are in season. Circle as many fruits as apply to you. Include fresh and canned fruit, but *do not* include dried fruit, fruit juices, fruit drinks, fruit bars or frozen fruit deserts.

| | | | | | |
|------------------------------------|-----------|-----------|-----------|--------|--------|
| Orange | Mandarin | Apple | Pear | Banana | Grapes |
| Strawberry | Kiwifruit | Apricot | Nectarine | Peach | Plum |
| Watermelon | Rockmelon | Pineapple | Mango | Pawpaw | |
| Any others? (please specify) _____ | | | | | |

Q79. From the following list, circle the vegetables which you eat *at least once a week* (on average), when they are in season. Circle as many vegetables as apply to you. Include all forms of vegetables, e.g. fresh, frozen, canned, salads.

| | | | | | |
|------------------------------------|-------------|------------|-----------------|-------------|--------------|
| Potato | Sweet corn | Green peas | Green beans | Baked beans | Dried beans |
| Lentils | Chick peas | Tomato | Carrot | Pumpkin | Sweet potato |
| Beetroot | Cucumber | Capsicum | Celery | Spinach | Silver beet |
| Cabbage | Cauliflower | Broccoli | Brussel sprouts | Onion | Asparagus |
| Mushroom | Sprouts | Avocado | Zucchini | Eggplant | Lettuce |
| Any others? (please specify) _____ | | | | | |

Q80. Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you **over the past week**. There are no right or wrong answers. Do not spend too much time on any one statement.

The rating scale is as follows:

0. Did not apply to me at all.
1. Applied to me to some degree, or some of the time.
2. Applied to me a considerable degree, or a good part of the time.
3. Applied to me very much, or most of the time.

| | | | | |
|--|---|---|---|---|
| 1. I found myself getting upset by quite trivial things. | 0 | 1 | 2 | 3 |
| 2. I just couldn't seem to get going. | 0 | 1 | 2 | 3 |
| 3. I had a feeling of faintness. | 0 | 1 | 2 | 3 |
| 4. I experienced breathing difficulties (eg. excessively rapid breathing, in the absence of physical exertion). | 0 | 1 | 2 | 3 |
| 5. I felt sad and depressed. | 0 | 1 | 2 | 3 |
| 6. I found it hard to calm down after something else. | 0 | 1 | 2 | 3 |
| 7. I perspired noticeably (eg. hands sweaty) in the absence of high temperatures or physical exertion. | 0 | 1 | 2 | 3 |
| 8. I found myself getting impatient when I was delayed in any way (eg. lifts, traffic lights, being kept waiting). | 0 | 1 | 2 | 3 |
| 9. I found myself in situations which made me so anxious I was most relieved when they ended. | 0 | 1 | 2 | 3 |
| 10. I tend to over-react to situations. | 0 | 1 | 2 | 3 |
| 11. I found myself getting upset rather easily. | 0 | 1 | 2 | 3 |
| 12. I felt that I had nothing to look forward to. | 0 | 1 | 2 | 3 |
| 13. I couldn't seem to experience any positive feelings at all. | 0 | 1 | 2 | 3 |
| 14. I found that I was very irritable. | 0 | 1 | 2 | 3 |
| 15. I was aware of dryness in my mouth. | 0 | 1 | 2 | 3 |
| 16. I felt that I had lost interest in just about everything. | 0 | 1 | 2 | 3 |
| 17. I could see nothing in the future to be hopeful about. | 0 | 1 | 2 | 3 |
| 18. I was aware of the action of my heart in the absence of physical exertion (eg. heart rate increase, missing a beat). | 0 | 1 | 2 | 3 |
| 19. I felt scared without any good reason. | 0 | 1 | 2 | 3 |
| 20. I felt that life wasn't worthwhile. | 0 | 1 | 2 | 3 |
| 21. I felt that I was rather touchy. | 0 | 1 | 2 | 3 |
| 22. I felt that I was using a lot of nervous energy. | 0 | 1 | 2 | 3 |
| 23. I couldn't seem to get enough enjoyment out of the things I did. | 0 | 1 | 2 | 3 |
| 24. I had a feeling of shakiness (eg. legs going to give way). | 0 | 1 | 2 | 3 |

The rating scale is as follows:

0. Did not apply to me at all.
1. Applied to me to some degree, or some of the time.
2. Applied to me a considerable degree, or a good part of the time.
3. Applied to me very much, or most of the time.

| | | | | |
|--|---|---|---|---|
| 25. I felt down-hearted and blue. | 0 | 1 | 2 | 3 |
| 26. I found it difficult to work up the initiative to do something. | 0 | 1 | 2 | 3 |
| 27. I found it hard to wind down. | 0 | 1 | 2 | 3 |
| 28. I was intolerant of anything that kept me from getting on with what I was doing. | 0 | 1 | 2 | 3 |
| 29. I had difficulty in swallowing. | 0 | 1 | 2 | 3 |
| 30. I feared that I could be "thrown" by some trivial but unfamiliar task. | 0 | 1 | 2 | 3 |
| 31. I felt I was pretty worthless. | 0 | 1 | 2 | 3 |
| 32. I was unable to become enthusiastic about anything. | 0 | 1 | 2 | 3 |
| 33. I was worried about situations in which I might panic and make a fool of myself. | 0 | 1 | 2 | 3 |
| 34. I was in a state of nervous tension. | 0 | 1 | 2 | 3 |
| 35. I felt I was close to panic. | 0 | 1 | 2 | 3 |
| 36. I felt I wasn't much as a person. | 0 | 1 | 2 | 3 |
| 37. I found it difficult to relax. | 0 | 1 | 2 | 3 |
| 38. I felt terrified. | 0 | 1 | 2 | 3 |
| 39. I experienced trembling (eg. in the hands). | 0 | 1 | 2 | 3 |
| 40. I found myself getting agitated. | 0 | 1 | 2 | 3 |
| 41. I felt that life was meaningless. | 0 | 1 | 2 | 3 |
| 42. I found it difficult to tolerate interruptions to what I was doing. | 0 | 1 | 2 | 3 |

Q81. Have any of the following happened to you in the last year?
(Please circle Yes or No for each item)

| | | |
|-----|----|---|
| Yes | No | Pregnancy problems |
| Yes | No | Death of a close relative - which relative? |
| Yes | No | Death of a close friend |
| Yes | No | Separation or divorce |
| Yes | No | Marital problems |
| Yes | No | Problems with your children |
| Yes | No | Your own job loss (not voluntary) |
| Yes | No | Your partner's job loss (not voluntary) |
| Yes | No | Money problems |
| Yes | No | Residential move |
| Yes | No | Other (please describe) |

| |
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| |

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Q82. Which words best describe your family's money situation?
(Please circle **one** answer only)

☐

- 0 We are spending more money than we get.
- 1 We have just enough money to get us through to the next pay day.
- 2 There's some money left over each week, but we just spend it.
- 3 We can save a bit every now and again.
- 4 We can save a lot.

The following 3 questions ask about your relationship with your partner. If you do not have a partner (live in or otherwise) please leave these questions and go to Q86.

Q83. Most people have disagreements in their relationships. Please indicate below the extent of agreement or disagreement between you and your partner for each of the following items.

| | Always Agree | Almost Always Agree | Occasionally Agree | Frequently Disagree | Almost Always Disagree | Always Disagree |
|--|--------------|---------------------|--------------------|---------------------|------------------------|-----------------|
| a. Philosophy of life. | 5 | 4 | 3 | 2 | 1 | 0 |
| b. Aims, goals and things believed to be important | 5 | 4 | 3 | 2 | 1 | 0 |
| c. Amount of time spent together. | 5 | 4 | 3 | 2 | 1 | 0 |

Q84. How often would you say the following events occur between you and your partner?

| | Never | Less than once a month | Once or twice a month | Once or twice a week | Once a day | More often |
|--|-------|------------------------|-----------------------|----------------------|------------|------------|
| a. Have a stimulating exchange of ideas. | 0 | 1 | 2 | 3 | 4 | 5 |
| b. Calmly discuss something. | 0 | 1 | 2 | 3 | 4 | 5 |
| c. Work together on a project. | 0 | 1 | 2 | 3 | 4 | 5 |

Q85. The numbers on the following lines represent different degrees of happiness in your relationship. The middle point, "happy", represents the degree of happiness of most relationships. Please circle the number which best describes the degree of happiness, all things considered, of your relationship.

| | | | | | | |
|-------------------|----------------|------------------|-------|------------|-----------------|---------|
| 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Extremely Unhappy | Fairly Unhappy | A little Unhappy | Happy | Very Happy | Extremely Happy | Perfect |

Q86. This is called the Family Assessment Device; it was developed to give an idea of how families work together. (Please circle **one** answer only for each item)

Item 1

Below are statements about families and family relationships. Circle the category which best describes your family - the people living in your house.

| | Strongly Agree | Agree | Disagree | Strongly Disagree |
|--|-------------------|-------|----------|----------------------|
| a. Planning family activities is difficult because we misunderstand each other | 3 | 2 | 1 | 0 |
| b. In times of crisis we can turn to each other for support | 3 | 2 | 1 | 0 |
| c. We cannot talk to each other about sadness we feel | 3 | 2 | 1 | 0 |
| d. Individuals (in the family) are accepted for what they are | 3 | 2 | 1 | 0 |
| e. We avoid discussing our fears and concerns | 3 | 2 | 1 | 0 |
| f. We express feelings to each other | 3 | 2 | 1 | 0 |
| g. There are lots of bad feelings in our family | 3 | 2 | 1 | 0 |
| h. We feel accepted for what we are | 3 | 2 | 1 | 0 |
| i. Making decisions is a problem in our family | 3 | 2 | 1 | 0 |
| j. We are able to make decisions about how to solve problems | 3 | 2 | 1 | 0 |
| k. We don't get on well together | 3 | 2 | 1 | 0 |
| l. We confide in each other | 3 | 2 | 1 | 0 |
| m. Drinking is a source of tension or disagreement in our family | 3 | 2 | 1 | 0 |

Item 2

The following list describes some of the ways people feel at different times. During the past few weeks, how often have you felt:

| | Always | Sometimes | Never |
|---|--------|-----------|-------|
| a. on top of the world? | 2 | 1 | 0 |
| b. very lonely or remote from other people? | 2 | 1 | 0 |
| c. particularly excited or interested in something? | 2 | 1 | 0 |
| d. depressed or very unhappy? | 2 | 1 | 0 |
| e. pleased about having accomplished something? | 2 | 1 | 0 |
| f. bored? | 2 | 1 | 0 |
| g. proud because someone complimented you on something? | 2 | 1 | 0 |
| h. so restless you couldn't sit long in a chair? | 2 | 1 | 0 |
| i. that things were going your way? | 2 | 1 | 0 |
| j. upset because someone criticised you? | 2 | 1 | 0 |

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Item 3

Taking things all together, how would you say things are for you these days?

☐

- 0 Not too happy
- 1 Reasonably happy
- 2 Very happy

Item 4

And how would you say things are for your spouse/partner?

☐

- 0 Not too happy
- 1 Reasonably happy
- 2 Very happy
- 3 No spouse/partner

The following questions ask about your friends and family with whom you communicate regularly.

Q87. How often do you have contact (including telephone) with members of your family, excluding those living with you?

☐☐☐

| | Child's Mother | Child's Father | Your Partner |
|-----------------------------|-------------------|-------------------|-----------------|
| Not at all | 0 | 0 | 0 |
| Less than monthly | 1 | 1 | 1 |
| Once or twice a month | 2 | 2 | 2 |
| Approximately once a week | 3 | 3 | 3 |
| More often than once a week | 4 | 4 | 4 |

Q88. How often do you have contact (including telephone) with friends, excluding those living with you?

☐☐☐

| | Child's Mother | Child's Father | Your Partner |
|-----------------------------|-------------------|-------------------|-----------------|
| Not at all | 0 | 0 | 0 |
| Less than monthly | 1 | 1 | 1 |
| Once or twice a month | 2 | 2 | 2 |
| Approximately once a week | 3 | 3 | 3 |
| More often than once a week | 4 | 4 | 4 |

Q89. Among these family and friends, how many people are there who you feel close to, and with whom you can talk frankly, without having to watch what you say?

☐☐☐

| | Child's Mother | Child's Father | Your Partner |
|--------------------|-------------------|-------------------|-----------------|
| None Go to Q91 | 0 | 0 | 0 |
| 1 – 2 people | 1 | 1 | 1 |
| 3 – 5 people | 2 | 2 | 2 |
| More than 5 people | 3 | 3 | 3 |

Q90. Do any of these people live within 10 minutes drive of you?

| | Child's Mother | Child's Father | Your Partner | <input type="text"/> | <input type="text"/> | <input type="text"/> |
|-----|-------------------|-------------------|-----------------|----------------------|----------------------|----------------------|
| No | 0 | 0 | 0 | | | |
| Yes | 1 | 1 | 1 | | | |

Section 3

These questions are mostly about your 13 year old study child.

Please write the answer in the space provided or circle the answer where applicable.

ALL ANSWERS ARE STRICTLY CONFIDENTIAL

Q91. On average, how much time do you spend with your child each day from Monday to Friday (Include the time you spend interacting with each other, helping with homework, talking and just 'being together' – excluding sleeping).

| | Child's Mother | Child's Father | Your Partner |
|--------------------|-------------------|-------------------|-----------------|
| None | 0 | 0 | 0 |
| Less than 1 hour | 1 | 1 | 1 |
| About 1 hour | 2 | 2 | 2 |
| About 1 to 2 hours | 3 | 3 | 3 |
| About 3 to 5 hours | 4 | 4 | 4 |
| More than 5 hours | 5 | 5 | 5 |

Q92. On average, how much time do you spend with your child each day in the weekend (Include the time you spend helping with homework, talking and interacting with each other – excluding sleeping).

| | Child's Mother | Child's Father | Your Partner |
|------------------|-------------------|-------------------|-----------------|
| None | 0 | 0 | 0 |
| Less than 1 hour | 1 | 1 | 1 |
| 1 to 5 hours | 2 | 2 | 2 |
| 6 to 10 hours | 3 | 3 | 3 |
| 11 to 20 hours | 4 | 4 | 4 |

Q93a. How much time does your child usually spend watching TV or videos?

- 0 None
- 1 Up to 1 hour a day (3 to 6 hrs a week)
- 2 Between 1 and 2 hours a day (7 to 13 hrs a week)
- 3 Between 2 and 3 hours a day (14 to 21 hrs a week)
- 4 4 hours or more a day (21 hrs or more a week)

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Q93b. How much time does your child usually spend using a computer (including Internet and chat use)? ☐

- 0 None
- 1 Up to 1 hour a day (3 to 6 hrs a week)
- 2 Between 1 and 2 hours a day (7 to 13 hrs a week)
- 3 Between 2 and 3 hours a day (14 to 21 hrs a week)
- 4 4 hours or more a day (21 hrs or more a week)

Q94. How would you compare the physical activity level of your child with that of other children of the same age? ☐

- 0 I am unable to make the comparison
- 1 My child is less active than other children
- 2 My child is as active as other children
- 3 My child is more active than other children

Q95. How does your child's level of activity now compare to 12 months ago? ☐

- 0 Less active than 12 months ago
- 1 About the same as 12 months ago
- 2 More active than 12 months ago

Q96. How would you rate the ability level of your child for each of the following skills?

| | | Poor | Below Average | Average | Above Average | Excellent |
|----|------------------|------|------------------|---------|------------------|-----------|
| a. | Running | 0 | 1 | 2 | 3 | 4 |
| b. | Jumping | 0 | 1 | 2 | 3 | 4 |
| c. | Hopping | 0 | 1 | 2 | 3 | 4 |
| d. | Skipping | 0 | 1 | 2 | 3 | 4 |
| e. | Throwing | 0 | 1 | 2 | 3 | 4 |
| f. | Catching | 0 | 1 | 2 | 3 | 4 |
| g. | Kicking | 0 | 1 | 2 | 3 | 4 |
| h. | Striking/hitting | 0 | 1 | 2 | 3 | 4 |
| i. | Dodging | 0 | 1 | 2 | 3 | 4 |
| j. | Biking | 0 | 1 | 2 | 3 | 4 |
| k. | Balancing | 0 | 1 | 2 | 3 | 4 |

Q97. What year/grade is your child in at school now? Year/Grade _____ ☐

Q98. Has your child ever repeated a year/grade at school? ☐

- 0 No
- 1 Yes Which year(s)/grade(s)? _____ ☐

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Q99. How satisfied are you with the standard of education offered at your child's current school?

- 0 Very dissatisfied
- 1 Dissatisfied
- 2 Neither satisfied or dissatisfied
- 3 Satisfied
- 4 Very satisfied

☐

Q100. How would you describe your child's academic performance in school during the past six months?

- 0 Poor
- 1 Below average
- 2 Average
- 3 Very good
- 4 Excellent

☐

Q101. How satisfied are you with your child's progress at school in the following areas:

| | Very Satisfied | Satisfied | Neither | Dissatisfied | Very Dissatisfied |
|--|----------------|-----------|---------|--------------|-------------------|
| a. Learning skills? | 4 | 3 | 2 | 1 | 0 |
| b. Physical development, coordination? | 4 | 3 | 2 | 1 | 0 |
| c. Getting on with other children? | 4 | 3 | 2 | 1 | 0 |
| d. General behaviour? | 4 | 3 | 2 | 1 | 0 |

Q102. Is your child limited in the kind or amount of school work he/she does because of physical problems?

0 No Go to Q104

1 Yes

☐

Q103. How long has your child been limited in this way?

- 0 < 6 months
- 1 6 months to 2 years
- 2 More than 2 years

☐

Office use only

Q104. Is your child limited in the kind or amount of school work he/she does because of emotional problems?

☐

0 No Go to Q106

1 Yes



Q105. How long has your child been limited in this way?

☐

0 < 6 months

1 6 months to 2 years

2 More than 2 years

Q106. Is your child limited in the kind or amount of school work he/she does because of learning problems?

☐

0 No Go to Q108

1 Yes



Q107. How long has your child been limited in this way?

☐

0 < 6 months

1 6 months to 2 years

2 More than 2 years

Q108. Is your child limited in the kind or amount of school work he/she does because of speech and/or language problems?

☐

0 No Go to Q110

1 Yes



Q109. How long has your child been limited in this way?

☐

0 < 6 months

1 6 months to 2 years

2 More than 2 years

Office use only

Q110. Has your child ever received any of the following types of special education or special teaching:

| | No | Yes, Full-time | Yes, Part-time |
|---|----|-------------------|-------------------|
| a. For children with visual or hearing difficulties? | 0 | 1 | 2 |
| b. For children with speech and/or language problems? | 0 | 1 | 2 |
| c. For children who are intellectually handicapped? | 0 | 1 | 2 |
| d. For children with emotional or behavioural problems? | 0 | 1 | 2 |
| e. For children who are intellectually gifted? | 0 | 1 | 2 |
| f. For children with remedial education needs? | 0 | 1 | 2 |

Q111. During the past six months has your child (or have you on your child's behalf) had contact with a school counsellor or guidance officer?

0 No

1 Yes How many times? _____

☐
☐ ☐

Q112. During the past six months has your child (or have you on your child's behalf) had contact with a teacher for a behavioural problem or a learning problem?

0 No

1 Yes How many times? _____

☐
☐ ☐

Q113. Does your child take part in any of the following activities outside of school hours:

| | No | Yes |
|--|----|-----|
| a. Organised groups such as scouts, guides, church groups? | 0 | 1 |
| b. Organised sport like football, netball, little athletics? | 0 | 1 |
| c. Informal sporting activities like swimming, rollerblading? | 0 | 1 |
| d. Music, art, drama, dance outside of school? | 0 | 1 |
| e. Informal recreation like going to the movies or swimming pool? | 0 | 1 |
| f. Going to friend's houses (any friends, not necessarily school friends)? | 0 | 1 |

Office use only

Q114. How satisfied are you with the opportunities that your child has to take part in activities outside school?

☐

- 0 Very dissatisfied
- 1 Dissatisfied
- 2 Neither satisfied or dissatisfied
- 3 Satisfied
- 4 Very satisfied

Q115. How would you rate the overall health of your child?

☐

- 0 Poor (seldom well)
- 1 So-so (he/she is ill as often as he/she is well)
- 2 OK, could be better (mostly well)
- 3 Excellent (nearly always well)

Q116. Is your child limited in any physical activities (eg. running, biking, climbing stairs, lifting, dressing) because of health problems?

☐

- 0 No Go to Q118
- 1 Yes
↓

Q117. How long has your child been limited in this way?

☐

- 0 < 6 months
- 1 6 months to 2 years
- 2 More than 2 years

Q118. On average, how many serves of fruit does your child have each week (One serve = one piece of fresh fruit, or a 30 gram pack of sultanas, or five dried apricots - do not count juice)?

☐

- 0 None
- 1 1 to 5
- 2 6 to 10
- 3 11 to 15
- 4 More than 15

Q119. On average, how many serves of vegetables does your child have each week (One serve = half a cup of vegetables, or salad, or beans/lentils)?

☐

- 0 None
- 1 1 to 5
- 2 6 to 10
- 3 11 to 15
- 4 More than 15

Office use only

Q120. On average, how many times does your child have a high fibre breakfast cereal each week (such as Weetbix, Mini-wheats, Just Right, Sustain, Weeties, muesli)?

- 0 Not at all
- 1 1 to 5 times
- 2 6 to 8 times
- 3 More than 8 times

☐

Q121. On average, how many muesli or health bars does your child have each week?

- 0 None
- 1 1 to 4
- 2 5 to 8
- 3 9 to 15
- 4 More than 15

☐

Q122. On average, how many slices of high fibre bread (wholemeal, multi-grain, high fibre white) does your child have each week?

- 0 None
- 1 1 to 5
- 2 6 to 10
- 3 11 to 15
- 4 More than 15

☐

Q123. On average, how many serves of rice or pasta does your child have each week (One serve = one cup)?

- 0 None
- 1 1 to 4
- 2 5 to 8
- 3 More than 8

☐

Office use only

Q124. Does your child have now, or has your child had in the past, any of the following health professional diagnosed medical conditions or health problems?
(Please circle all appropriate answers)

| | No | Yes- In the past | Yes- Now | Yes-Now and In the past |
|--|----|---------------------|-------------|----------------------------|
| a. Anxiety problems | 0 | 1 | 2 | 3 |
| b. Arthritis or joint problems | 0 | 1 | 2 | 3 |
| c. Asthma | 0 | 1 | 2 | 3 |
| d. Attentional problems | 0 | 1 | 2 | 3 |
| e. Back pain | 0 | 1 | 2 | 3 |
| f. Behavioural problems | 0 | 1 | 2 | 3 |
| g. Chronic respiratory or breathing problems (other than asthma) | 0 | 1 | 2 | 3 |
| h. Co-ordination or clumsiness difficulties | 0 | 1 | 2 | 3 |
| i. Depression | 0 | 1 | 2 | 3 |
| j. Hay fever or some other allergy | 0 | 1 | 2 | 3 |
| k. Hearing impairment or deafness | 0 | 1 | 2 | 3 |
| l. Heart condition | 0 | 1 | 2 | 3 |
| m. Intellectual disability | 0 | 1 | 2 | 3 |
| n. Learning problems | 0 | 1 | 2 | 3 |
| o. Migraine or severe headache? | 0 | 1 | 2 | 3 |
| p. Neck pain | 0 | 1 | 2 | 3 |
| q. Sleep disturbance | 0 | 1 | 2 | 3 |
| r. Speech and/or language problems | 0 | 1 | 2 | 3 |
| s. Vision problems | 0 | 1 | 2 | 3 |
| t. Any other medical condition or health problem not mentioned above | 0 | 1 | 2 | 3 |

Q125. If you have answered "Yes" to any of the above, or have any other health professional diagnosed problem or condition please describe the condition or problem below in more detail (eg. is longsighted - wears glasses for reading; diagnosed with Attention Deficit Disorder; asthma requiring occasional medication)
(Please list every medical condition/health problem separately - otherwise leave blank)

☐

| | | | | | |
|-------|--|--|--|--|--|
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| | | | | | |

Q126. Has your child had any accidents or injuries since the last follow-up at ten years of age which required you to take him/her to a doctor (GP), hospital or clinic?

0 No Go to 127

☐

1 Yes (Please describe the accident, the injury, and any treatment.
e.g. fell off bike, cut arm, 3 stitches)

(Please list every accident/injury separately)

.....
.....
.....
.....

| | | | | | |
|--|--|--|--|--|--|
| | | | | | |
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| | | | | | |

Q127. Has your child been admitted to a hospital since the last follow-up at ten years of age?

0 No Go to Q128

☐

1 Yes

(Please list each admission separately)

which hospital? date?

what for?.....

which hospital? date?

what for?.....

which hospital? date?

what for?.....

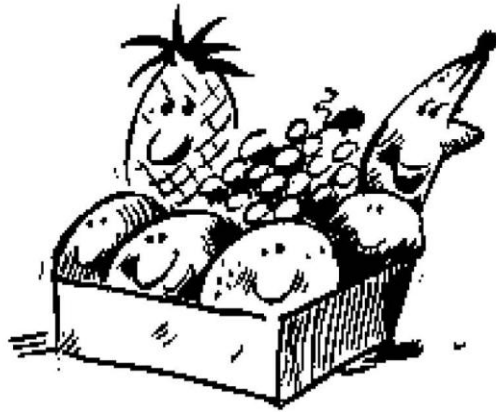
which hospital? date?

what for?.....

| | | | | | |
|--|--|--|--|--|--|
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Appendix 4 – Diet for 13 Years Old

ADOLESCENT'S DIETARY BOOKLET



Dear Parent

Thank you for agreeing to take part in this study. We are asking you to complete a food questionnaire for your adolescent (with his or her help) to help us answer important questions about how the things young people eat affect their health.

We realise that young person's eating habits can be extremely variable, but we would like you, as far as possible, to fill out the booklet as it relates to your adolescent's usual eating patterns over the past year. If your adolescent has made any major changes in the past two months, disregard these.

Please look at the examples over the page before filling out the rest of the questionnaire.

When you have completed the questionnaire, please return it to us. As soon as the results are available we will mail you a free assessment of your adolescent's diet.

If you have any problems with the booklet please ring the study research nurse on 9489 7796.

Thanks once again for your involvement and assistance!

YOUR ADOLESCENT'S EATING HABITS

This section is about the kinds of foods your young person usually eats. On the next few pages you will find lists of foods, separated by questions about your adolescent's eating habits.

Read through each list of foods and record about how often your adolescent usually eats these foods. We realise that your adolescent's food intake may vary from time to time, so just try to give us the best overall picture of what he/she eats that you can.

We are interested in **YOUR ADOLESCENT'S** eating habits, not that of someone else in your household.

THIS IS HOW TO ANSWER

We are going to ask you "About how often does your adolescent usually eat these foods?" Use the following simple code to write your answer in the space next to each food.

| | | |
|--|-------|----------|
| If your adolescent NEVER has a food | write | N |
| If your adolescent RARELY has a food (less than once a month) | write | R |

If your adolescent usually eats a food

| | | |
|---|-------|--------------------------|
| About once a MONTH | write | 1M |
| About twice a MONTH | write | 2M |
| About three times a MONTH | write | 3M |
| | | |
| About once a WEEK | write | 1W |
| About twice a WEEK | write | 2W |
| About three times a WEEK | write | 3W |
| and so on | | (4W, 5W, 6W) |
| | | |
| About once a DAY | write | 1D |
| About twice a DAY | write | 2D |
| and so on | | (3D, 4D, 5D, etc) |

Standard Serves

Alongside each food there is a "standard serve" size. The "standard" serve is not necessarily a "normal" serve, it is simply there to help us measure food intake. If your adolescent usually eats more or less than the standard serve size for a particular food, please indicate on the **COMMENTS** line what amount is usually eaten.

For example, if when your adolescent eats icecream he/she has one "scoop" instead of our "standard" serve of two "scoops", indicate how often icecream is eaten, and then write "one scoop only" on the comments line.

On the opposite page you will see some examples of how to fill out the questionnaire. Please read these carefully before you start to fill out the answers for your adolescent's diet.

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

HERE ARE SOME EXAMPLES

| | <u>STANDARD SERVE</u> | | <u>COMMENTS</u> |
|-------------------|---------------------------------|-------|-----------------|
| Custard | 1/2 cup | | _____ |
| Boiled egg | 1 egg | | _____ |
| Cucumber | 3 slices (each 0.5 cm thick) | | _____ |
| Tea | 1 cup | | _____ |
| Beetroot - canned | 2 slices | | _____ |

The child above has, on average :-

- A standard serve of custard **three times a week**
- Two boiled eggs **three times a month**
- Rarely eats cucumber
- Four cups of tea **every day**
- Half a standard serve (1 slice) of beetroot - canned,
twice a month

We realise that some parents have an exact idea of how often their adolescent eats particular foods, whilst others only have an approximate idea. Be as accurate as you can but do not spend too much time choosing your answers.

PLEASE GIVE AN ANSWER FOR EVERY FOOD

HOW TO ANSWER

| NEVER N | RARELY R | Times a MONTH $\frac{1}{2}$ M | Times a WEEK $\frac{1}{2}$ W and so on | Times a DAY $\frac{1}{2}$ D and so on |
|-------------------|--------------------|--|--|---|
|-------------------|--------------------|--|--|---|

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| | <u>CEREALS</u> | <u>COMMENTS</u> |
|--|----------------|-----------------|
| Porridge/Oatmeal 1 cup (cooked) | | |
| Muesli 1/2 cup | | |
| Other breakfast cereal 1 cup | | |
| Plain bran (raw) 1 tablespoon | | |
| Wheatgerm 1 tablespoon | | |
| Bread roll 1 roll (NOT hamburger buns) | | |
| Fried rice 1 cup (cooked) | | |
| Boiled rice 1 cup (cooked) | | |
| Instant noodles (Maggi etc.) 1 cup (cooked) | | |
| Other pasta 1 cup (cooked) (spaghetti, macaroni etc.) | | |

Q-1 How many slices of bread does your adolescent **usually** eat? **Remember the bread in toast and sandwiches.** If bread is not eaten at all, write 'none'.

..... slices/day OR slices/week

Q-2 What type of bread does your adolescent **usually** eat? (Circle the number beside one answer)

- 1 Wholemeal or mixed grain
- 2 White
- 3 About half the time wholemeal and half white
- 4 Other breads (e.g. rye, Hi-Fibe)
(please specify type)
- 5 My adolescent does not eat bread

Q-3 Does your adolescent eat **low-salt** types of bread? (Circle **one** answer)

ALL or MOST OF THE TIME OCCASIONALLY RARELY/NEVER

40

Q-4 Which of the following does your adolescent **usually** spread on bread or crackers? (Circle one answer)

- 1 Butter
- 2 Polyunsaturated margarine : please name
- 3 Table or cooking margarine; please name
- 4 Reduced-fat margarine (e.g. Era, Becel Light) please name
- 5 Dripping/Lard
- 6 My adolescent does not use anything
- 7 My adolescent does not eat bread or crackers
- 8 Something else : please name

Q-5 For whatever spread your adolescent has, is it **usually** the regular variety or reduced salt?
(Circle **one** answer)

- | | | | |
|---|---------------------------------|---|--------------------------------------|
| 1 | Usually has the regular variety | 2 | Usually has the reduced-salt variety |
|---|---------------------------------|---|--------------------------------------|

Q-6 What types of breakfast cereals does your adolescent most commonly eat?

Please name :

Q-7 If your adolescent eats **muesli** is it : (Circle **one** answer)

- | | | | |
|---|------------------|---|---------------------|
| 1 | Home made muesli | 2 | Pre-packaged muesli |
|---|------------------|---|---------------------|

Q-8 How many cups of milk does your adolescent **usually** add to breakfast cereal, porridge or muesli ? (Circle the number closest to the amount he/she has)

- 1 None
2 About a half a cup
3 About one cup
4 About one and a half cups
5 About two cups or more (please state how much)

Q-9 What type of milk does your adolescent **usually** add to cereals, porridge or muesli? (e.g. whole milk, Hi-Lo, Lite, skim, powdered skim, Shape, Farmers Best, goat's milk, condensed/evaporated milk etc.)?

Type of milk added :

Q-10 How many teaspoons of sugar or honey does your adolescent **usually** add to cereal, porridge or muesli. (Note : 1 level dessertspoon = 2 level teaspoons)

Write the number of level teaspoons your adolescent has here :

Q-11 Does your adolescent **add** salt to porridge? (Circle **one** number)

- 1 Yes
2 No
3 He/she doesn't eat porridge

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

CEREAL FOODS

COMMENTS

| | | | |
|---------------------------------|---------------------------------------|-------|-------|
| Crumpet or Muffin | 1 | | _____ |
| Croissant | 1 | | _____ |
| Fruit Loaf/Currant bread | 1 slice | | _____ |
| Sweet bun/doughnut | 1 | | _____ |
| Crispbread/Cracker | 2 | | _____ |
| Salted biscuits | 3 | | _____ |
| Plain sweet biscuits | 2 | | _____ |
| Fancy biscuits (eg choc-coated) | 2 | | _____ |
| Cake | 1 small cake or 1 slice large cake | | _____ |
| Milk pudding (eg rice, sago) | 1/2 cup | | _____ |
| Steamed sponge - suet | 1/4 small pudding | | _____ |

Q-1 Does your adolescent have milk :

(Circle one for each)

| | | | |
|-----------------------|-----|----|----------------------------------|
| in tea? | YES | NO | DOES NOT DRINK TEA |
| in coffee? | YES | NO | DOES NOT DRINK COFFEE |
| in coffee substitute? | YES | NO | DOES NOT DRINK COFFEE SUBSTITUTE |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY HAVE THESE DRINKS?

| <u>BEVERAGES</u> | | <u>COMMENTS</u> | |
|---|-----------------------------|-----------------|--|
| Sustagen (made with powder) | 1 cup | | |
| Sustagen Gold | small carton (300 ml) | | |
| Carton of other flavoured milk (eg chocolate, strawberry etc) | small carton (300 ml) | | |
| Cocoa | 1 cup | | |
| Drinking Chocolate/Milo/Quik etc. | 1 cup | | |
| Akta-Vite | 1 cup | | |
| Glass of milk (as such) | 1 glass | | |
| Milk shake/Thick shake | regular size | | |
| Tea | 1 cup | | |
| Herbal tea | 1 cup | | |
| Instant coffee | 1 cup | | |
| Ground coffee (eg filter/drip) | 1 cup | | |
| Decaffeinated coffee | 1 cup | | |
| Coffee substitute (eg Caro) | 1 cup | | |

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Q-2 Does your adolescent have cocoa/chocolate/Milo/Akta-Vite with : (Circle **one** number)

- 1 Mostly milk?
- 2 Mostly water?
- 3 About half and half?
- 4 He/she does not drink these drinks.

Q-3 What type of milk does your adolescent **usually** add to tea/coffee/cocoa/chocolate etc? (Please state the type of milk used eg whole milk, Lite, Hi-Lo, skim, powdered skim, Shape, Farmers Best, goats milk, condensed milk, evaporated milk etc.)

Type of milk added

Q-4 How many **teaspoons** of sugar/honey does your adolescent **usually** have in each cup of :

(Circle **one** number for each drink)

| | | | | | | | |
|----------------------|---|---|---|---|---|---|---|
| Tea? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Coffee? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Coffee substitute? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Cocoa? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Milo/Quik/Chocolate? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

HOW TO ANSWER

| NEVER N | RARELY R | Times a MONTH $\frac{1}{2}_3$ M | Times a WEEK $\frac{1}{2}_3$ W and so on | Times a DAY $\frac{1}{2}_3$ D and so on |
|-------------------|--------------------|--|--|---|
|-------------------|--------------------|--|--|---|

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>DAIRY PRODUCTS and EGGS</u> | | | <u>COMMENTS</u> | |
|--|------------------------|--------|-----------------|-------|
| Cheese | 30 grams (1 slice) | | | |
| Low-fat cottage cheese | 100 gm (1/2 carton) | | | |
| Cream | 1 tablespoon | | | |
| Yoghurt | 200 gm (1 carton) | | | |
| Icecream (from a tub) | 2 scoops | SUMMER | | |
| | | WINTER | | |
| Icecream desserts (eg Symphony, Vienetta) | 1 serving | SUMMER | | |
| | | WINTER | | |
| Icecream (on a stick/cone) | 1 icecream | SUMMER | | |
| | | WINTER | | |
| Vitari | 1 cone | SUMMER | | |
| | | WINTER | | |
| Ice block/Icy Pole | 1 | SUMMER | | |
| | | WINTER | | |
| Custard | 1/2 cup | | | |
| Fried egg | 1 egg | | | |
| Boiled egg | 1 egg | | | |
| Omelette/Scrambled eggs | 2 eggs | | | |

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Q-1 When your adolescent eats cheese, does he/she have the **reduced-salt** varieties (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Rarely or never
- 4 He/she does not eat cheese

Q-2 When your adolescent eats cheese, does he/she have the **reduced-fat** varieties (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Rarely or never
- 4 He/she does not eat cheese

Q-3 When your adolescent eats yoghurt which type is it? (Circle **one** number)

- 1 Plain (eg not fat-reduced)
- 2 Plain, low fat
- 3 Fruit flavoured (not fat-reduced)
- 4 Fruit flavoured, low-fat
- 5 Frozen yoghurt
- 6 He/she does not eat yoghurt

Q-4 When your adolescent eats ice-cream, diet-ice or similar is it **usually**? (Circle **one** number)

- 1 Low calorie
- 2 Regular icecream
- 3 Other (please state)

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.....

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>MEATS</u> | | <u>COMMENTS</u> |
|---|---|-----------------|
| Steak (eaten as such) | 1 medium (100g) (approximately size of fillet) | |
| Pork chop | 1 medium chop | |
| Lamb chop (loin chop size) | 2 chops | |
| Roast pork/pork fillet | 2 slices | |
| Roast beef/veal | 2 slices | |
| Roast lamb | 2 slices | |
| Sausages | 2 thick or 3 thin | |
| Frankfurters/Saveloys | 2 thick or 3 thin | |
| Bacon | 2 rashers | |
| Ham | 3 thin or 2 thick slices | |
| Luncheon meat/Fritz/ Devon/Windsor etc. | 3 slices (1 cm thick if small nob) | |
| Continental Sausage (salami/Mettwurst etc) | 3 slices | |
| Pate/liver paste | 1 tablespoon | |
| Liver | 1/2 liver (150 gm) | |
| Kidney | 2 kidneys | |
| Brains | 1/2 cup | |
| Pureed meat dishes (canned/bottled) | 1/2 cup | |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>MIXED DISHES</u> | | <u>COMMENTS</u> | |
|---|---|-----------------|-------|
| Hamburger WITH bun | 1 medium | | _____ |
| Hamburger patty WITHOUT bun | 1 medium | | _____ |
| Pizza (frozen) | 1 mini or 1/4 large | | _____ |
| Pizza (homemade or take-away) | 1/2 small or 1/4 large | | _____ |
| Sausage roll | 1 large or 2 small | | _____ |
| Meat pie | 1 individual | | _____ |
| Meat pie (homemade) | 1 individual or 1 slice of large pie | | _____ |
| Pastie | 1 individual | | _____ |
| Crumbed veal (schnitzel) | 1 large piece | | _____ |
| Stew/casserole/curry/goulash (with meat or chicken) | 1 cup | | _____ |
| Stew/casserole/curry/goulash (without meat or chicken) | 1 cup | | _____ |
| Chinese meat and veg dish | 1 cup | | _____ |
| Savoury pies/pastries (eg quiche) | 1 individual OR 1 slice of large pie | | _____ |
| Mince meat (eaten as such) | 1 cup | | _____ |
| Mince meat dishes (eg Shepherds' pie) | 1 piece (8x8x4cm) | | _____ |
| Spicy mince added to pastas (eg spag. sauce) | 1/2 cup mince | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| CHICKEN, FISH AND SEAFOOD | | COMMENTS |
|---|---|----------|
| Roast/Barbecue chicken | 2 slices of breast or 1 drumstick or 2 wings | |
| Boiled chicken | as above | |
| Crumbed, fried chicken | 4 small pieces | |
| Chicken nuggets | 6 nuggets | |
| Fish – fried (state what type of fish) | 1 piece or 6 nuggets | |
| Fish without batter (steamed, grilled/boiled) (please state what type of fish) | 1 piece | |
| Canned fish (tuna, salmon etc) | 1/3 cup | |
| Fish Fingers | 3 - 4 fingers | |
| Seafood (prawns, crab, lobster etc) | 1/2 cup | |
| Mornay dishes | 1 cup | |

Q-1 If your adolescent eats the following meats, how are they **usually** cooked? (Circle **one** for each food)

| | | | | |
|----------|-------|---------------|------------|-----------|
| Steak | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Chops | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Sausages | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Bacon | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |

Q-2 When your adolescent eats meat with fat on it, does he/she eat : (Circle **one** number)

- 1 All of the fat
- 2 Most of the fat
- 3 About half of the fat
- 4 Little or none of the fat
- 5 He/she does not eat meat

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Q-3 Does your adolescent have the skin removed from their chicken? (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes (about half the time or less)
- 3 Rarely (less than a quarter of the time)
- 4 Never
- 5 He/she does not eat chicken

Q-4 If your adolescent eats fried fish, in which of the following is it **usually** coated? (Circle **one** number)

- 1 Batter
- 2 Breadcrumbs
- 3 Flour
- 4 Other coating; please name.....
- 5 Fried without coating

Q-5 When your adolescent eats fish coated in batter, crumbs etc how often is it : (Circle **one for each**)

| | | | | |
|---------------------------------------|--------|-----------|--------|-------|
| Coated at home | ALWAYS | SOMETIMES | RARELY | NEVER |
| Pre-packed, frozen cooked at home | ALWAYS | SOMETIMES | RARELY | NEVER |
| Bought ready cooked from fish shop | ALWAYS | SOMETIMES | RARELY | NEVER |

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Q-6 If you buy fresh fish for your adolescent, what variety is it usually?

.....

HOW TO ANSWER

| NEVER N | RARELY R | Times a MONTH $\frac{1}{2}$ M | Times a WEEK $\frac{1}{2}$ W and so on | Times a DAY $\frac{1}{2}$ D and so on |
|-------------------|--------------------|--|--|---|
|-------------------|--------------------|--|--|---|

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>CANNED and DRIED VEGETABLES</u> | | | <u>COMMENTS</u> |
|--|------------------|-------|-----------------|
| Potato - canned | 2-3 small | | _____ |
| Potato - packet (powdered) | 1/3 cup (cooked) | | _____ |
| Potato salad | 1/3 cup | | _____ |
| Carrots - canned | 1/3 cup | | _____ |
| Beetroot - canned | 2 slices | | _____ |
| Green beans - canned | 1/3 cup | | _____ |
| Haricot, Lima beans - canned | 1/3 cup | | _____ |
| Baked beans in tomato sauce | 1/3 cup | | _____ |
| Green peas - canned | 1/3 cup | | _____ |
| Lentils - dried/canned | 1/3 cup | | _____ |
| Zucchini salad | 1/3 cup | | _____ |
| Sweetcorn - canned (including creamed corn) | 1/3 cup | | _____ |
| Mushrooms - canned | 6-7 small ones | | _____ |
| Mushrooms - canned in sauce | 1/3 cup | | _____ |
| Olives | 3 medium | | _____ |
| Gherkins/Pickled onions | 3 pieces | | _____ |
| Pureed vegetables (canned/bottled) | 1/3 cup | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|----------|----------|-------------------------------------|--|--|
| N | R | $\frac{1}{2}$ ₃ M | $\frac{1}{2}$ ₃ W and so on | $\frac{1}{2}$ ₃ D and so on |

The following list of foods contains some vegetables that may be eaten much more frequently at some times of the year than others (eg in the warmer or cooler weather). Please fill in how often each food is eaten in **BOTH** the warmer months of the year (**SUMMER**) and the cooler months (**WINTER**).

For example :- If your adolescent usually has :

A standard serve of peas about **twice a week** during the **warmer** months of the year and about **every day** during the **cooler** months :

and:

Two medium potatoes (roasted) a **week** throughout the **year** :

You would write :

| | | <u>Summer</u> | <u>Winter</u> | |
|-------------------------|----------|---------------|---------------|-------|
| Green peas | 1 cup | | | _____ |
| Potato - roasted | 1 medium | | | _____ |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>SEASONAL VEGETABLES</u> | | <u>Summer</u> | <u>Winter</u> | |
|--|-------------|---------------|---------------|-------|
| Potato - fresh & mashed (with milk) | 1/3 cup | | | _____ |
| Potato - fresh, boiled | 1 medium | | | _____ |
| Potato - roasted | 1 medium | | | _____ |
| French fries/hot chips | 17-18 chips | | | _____ |
| Potato Gems/ Pommes Noisettes | about 5 | | | _____ |
| Carrots (fresh/frozen) | 1/3 cup | | | _____ |
| Turnip/Swede (fresh/frozen) | 1/3 cup | | | _____ |
| Broad beans (fresh/frozen) | 1/2 cup | | | _____ |
| Green beans (fresh/frozen) | 1/3 cup | | | _____ |

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| <u>SEASONAL VEGETABLES (continued)</u> | | <u>Summer</u> | <u>Winter</u> |
|--|------------------------------|----------------------|----------------------|
| Green peas (fresh/frozen) | 1/3 cup | | |
| Cabbage | 1/3 cup | | |
| Brussels sprouts (fresh/frozen) | 5 - 6 | | |
| Silver beet/spinach (fresh/frozen) | 1/3 cup | | |
| Broccoli (fresh/frozen) | 1/3 cup | | |
| Cauliflower (fresh/frozen) | 1/2 cup | | |
| Pumpkin | 1/3 cup | | |
| Sweetcorn (fresh/frozen) | 1 small cob | | |
| Zucchini (courgettes) | 1 medium sized | | |
| Onion - fried | 1/4 cup | | |
| Onion (raw, baked, boiled) (fresh/frozen) | 1 medium | | |
| Tomato - fresh | 1 medium | | |
| Tomato - grilled/fried | 1/2 medium | | |
| Lettuce | 2 small leaves | | |
| Cucumber | 3 slices (each 0.5 cm thick) | | |
| Coleslaw | 1/2 cup | | |
| Celery (fresh/frozen) | 1 x 15cm stick | | |
| Capsicum (Green pepper) (fresh/frozen) | 2 strips (each 0.5 cm thick) | | |
| Mushrooms - fresh | 6-7 small ones | | |
| Sprouted bean shoots | 1/3 cup | | |
| Fried mixed vegetables (eg stir fried) | 1/2 cup | | |

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Q-1 When you use canned vegetables, are they **reduced-salt** varieties? (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Never or rarely
- 4 Only for some vegetables (please state which.....)

Q-2 Is salt added to the cooking water when boiling the following foods? (Circle **one for each food**)

| | | | |
|----------------|---------|-----------|-------|
| Vegetables | USUALLY | SOMETIMES | NEVER |
| Pasta and rice | USUALLY | SOMETIMES | NEVER |

Q-3 If salt is added to the cooking water when boiling foods, is the water : (Circle **one** number)

- 1 Lightly salted
- 2 Medium salted
- 3 Heavily salted
- 4 Salting is highly varied
- 5 Salt is not added to cooking water

Q-4 How often do you add salt to your adolescent's meals **after** they are cooked? (Circle **one** number)

- 1 Rarely or never
- 2 Sometimes
- 3 Always or nearly always

Q-5 When you add salt at the table to your adolescent's meals, how much is **usually** added?
(Circle **one** number)

- 1 A light sprinkle
- 2 A medium sprinkle
- 3 A heavy sprinkle
- 4 Salting is highly varied
- 5 Salt is not added at the table

Q-6 When you cook vegetables for your adolescent which of the following methods is the one **most commonly** used? (Circle **one** number)

- 1 Boiled in a little water
- 2 Boiled in a lot of water
- 3 Steamed
- 4 Cooked in a pressure cooker
- 5 Microwaved
- 6 Stir-fried

HOW TO ANSWER

| | | | | |
|--------------|---------------|--------------------------|-------------------------|------------------------|
| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W | $\frac{1}{2}$ D |
| | | | and so on | and so on |

The fruits listed below are only available for a short time during the year. Therefore we only want you to record how often your adolescent has them when they are IN SEASON.

HOW OFTEN DOES YOUR ADOLESCENT EAT THESE FOODS WHEN THEY ARE IN SEASON?

| <u>SEASONAL FRUITS</u> | | <u>COMMENTS</u> | |
|------------------------|---------------|-----------------|-------|
| Berries - fresh/frozen | 3/4 cup | | |
| Melon (not watermelon) | 1 large slice | | |
| Peach - fresh | 1 medium | | |
| Plum - fresh | 3-4 plums | | |
| Nectarine - fresh | 1 medium | | |
| Apricot - fresh | 3 apricots | | |
| Grapes - fresh | about 20 | | |
| Pineapple - fresh | 1 slice | | |
| Avocado | 1/2 a medium | | |

Please list here, along with your adolescent's standard serve size, any other fruit that your adolescent eats (eg mango; pureed, canned/bottled fruits)

| | | | |
|--|--|--|--|
| | | | |
| | | | |

HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>NUTS and SNACKS</u> | | <u>COMMENTS</u> | |
|--|----------------------------------|-----------------|-------|
| Potato crisps, Twisties etc | 1 small bag or 14 - 15 pieces | | |
| Peanuts (fresh) | 9 - 10 nuts | | |
| Nuts - salted & cooked | 9 - 10 nuts | | |
| Other unsalted nuts (fresh walnuts/almonds etc) | 5 - 6 nuts | | |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ / ₂ / ₃ M | ¹ / ₂ / ₃ W and so on | ¹ / ₂ / ₃ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>SOUPS</u> | | <u>COMMENTS</u> | |
|-------------------------------|-------|-----------------|-------|
| Canned soup (eaten as such) | 1 cup | WINTER | |
| | | SUMMER | |
| Packet soup (eaten as such) | 1 cup | WINTER | |
| | | SUMMER | |
| Homemade soup (eaten as such) | 1 cup | WINTER | |
| | | SUMMER | |

Write an example of the type of soup your adolescent most often eats (eg canned tomato; homemade pea and ham)

.....

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY EAT THESE FOODS?

| <u>CONFECTIONERY, JAMS AND SAUCES</u> | | | <u>COMMENTS</u> |
|---|---------------------------|-------|-----------------|
| Chocolate | 1 small bar (50 grams) | | _____ |
| Chocolate covered bar (eg Mars/Bounty) | 1 bar | | _____ |
| Individually wrapped lollies, eg toffees | 4 - 5 lollies | | _____ |
| Packet lollies (eg Lifesavers/Polos) | 1 small packet | | _____ |
| Muesli bar/Health bar | 1 bar | | _____ |
| Honey, jam, marmalade | 1 tablespoon | | _____ |
| Vegemite, marmite etc | 1/2 teaspoon | | _____ |
| Thick sauces (tomato/HP etc) | 1tablespoon | | _____ |
| Polyunsaturated Mayonnaise/ Salad cream | 1 tablespoon | | _____ |
| Regular Mayonnaise/ Salad cream | 1 tablespoon | | _____ |
| Low calorie salad dressings | 1 tablespoon | | _____ |
| Polyunsaturated salad dressings | 1 tablespoon | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|------------------|------------------------------|------------------------------|
| N | R | $\frac{1}{2}$ M | $\frac{1}{2}$ W and so on | $\frac{1}{2}$ D and so on |

ABOUT HOW OFTEN DOES YOUR ADOLESCENT USUALLY HAVE THESE FOODS?

| <u>BEVERAGES</u> | | <u>COMMENTS</u> |
|---|---------------------|-----------------|
| Glass of cordial | medium glass | _____ |
| Glass of cola (eg Coca Cola) | medium glass | _____ |
| Glass of fizzy drink Includes mineral water with juice | medium glass | _____ |
| Glass of low-calorie fizzy drink | medium glass | _____ |
| Fruit drink (eg Fruit Box) | 250 ml carton | _____ |
| Pure fruit juice | medium glass | _____ |
| Vegetable juice | small glass | _____ |
| Water/Spring water | medium glass | _____ |
| Mineral Water | medium glass | _____ |

OTHER FOODS COMMENTS
 PLEASE LIST ANY OTHER FOODS HERE AND ON THE NEXT PAGE THAT YOUR ADOLESCENT HAS EATEN,
 PARTICULARLY TYPES OF WHOLE GRAINS, NUTS, SEEDS AND FISH

| | | |
|-------|-------|-------|
| | | _____ |
| | | _____ |
| | | _____ |
| | | _____ |
| | | _____ |

If your adolescent has any other foods or drinks that we have not mentioned, at least once a month, please write them down here and tell us how often he/she has them, using the same response scale as before (eg 1D, 3M etc).

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ / ₂ / ₃ M | ¹ / ₂ / ₃ W and so on | ¹ / ₂ / ₃ D and so on |

FOODS AND DRINKS MY ADOLESCENT CONSUMES THAT HAVE NOT BEEN MENTIONED :

| Name of Food | His/her usual serve size | How often is it eaten? |
|--------------|-----------------------------|---------------------------|
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |

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VITAMIN AND MINERAL SUPPLEMENTS

If your adolescent takes any vitamins or minerals, or any other dietary supplements, such as fibre tablets, lecithin, kelp, yeast etc, please fill in the table below. (Check the label on the box or bottle if you are unsure of some of the answers).

| BRAND (eg Nyal) | NAME OF PRODUCT (eg vitamin C pill) | SIZE OF DOSE (eg 250 mg) | NUMBER OF DOSES (eg 2 per day) |
|--------------------|--|-----------------------------|-----------------------------------|
| _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ |

WHAT WAS YOUR ADOLESCENT'S WEIGHT AND LENGTH AT BIRTH

WAS YOUR ADOLESCENT BREAST FED? (Please circle answer) YES NO

IF SO, FOR HOW LONG WAS YOUR ADOLESCENT BREAST FED?

HAS YOUR ADOLESCENT CHANGED HIS/HER DIET IN PAST 2-3 MONTHS? YES NO

IF SO, WHAT ARE THESE CHANGES?

.....

THANK YOU VERY MUCH FOR YOUR PARTICIPATION IN THIS STUDY

REMEMBER

**PLEASE RETURN THIS QUESTIONNAIRE
BEFORE LEAVING TODAY
OR
AS SOON AS POSSIBLE**

**We would welcome any comments you may have regarding
this questionnaire below**

Appendix 5 – Diet Used in 8 Years Old

Dear Parent

Thank you for agreeing to take part in this study. We are asking you to complete a food questionnaire for your child (with his or her help) to help us answer important questions about how the things children eat affect their health.

We realise that children's eating habits can be extremely variable, but we would like you, as far as possible, to fill out the booklet as it relates to your child's usual eating patterns over the past year. If your child has made any major changes in the past two months, disregard these.

Please look at the examples over the page before filling out the rest of the questionnaire.

When you have completed the questionnaire, please return it in the reply paid envelope. As soon as the results are available we will mail you a free assessment of your child's diet.

If you have any problems with the booklet please ring the study research nurse on 9340 8463.

Thanks once again for your involvement and assistance!

PLEASE SIGN HERE :

I give my consent for this information about my child's diet to be used in a research study of diet and asthma. I understand that the information is confidential and that I can withdraw my consent at any time.

Signature : Date :
(Parent or guardian)

YOUR CHILD'S EATING HABITS

This section is about the kinds of foods your child usually eats. On the next few pages you will find lists of foods, separated by questions about your child's eating habits.

Read through each list of foods and record about how often your child usually eats these foods. We realise that your child's food intake may vary from time to time, so just try to give us the best overall picture of what your child eats that you can.

We are interested in **YOUR CHILD'S** eating habits, not that of someone else in your household.

THIS IS HOW TO ANSWER

We are going to ask you "About how often does your child usually eat these foods?" Use the following simple code to write your answer in the space next to each food.

If your child **NEVER** has a food write **N**
If your child **RARELY** has a food (less than once a month) write **R**

If your child usually eats a food

About **once** a **MONTH** write **1M**
About **twice** a **MONTH** write **2M**
About **three** times a **MONTH** write **3M**

About **once** a **WEEK** write **1W**
About **twice** a **WEEK** write **2W**
About **three** times a **WEEK** write **3W**
and so on (**4W, 5W, 6W**)

About **once** a **DAY** write **1D**
About **twice** a **DAY** write **2D**
and so on (**3D, 4D, 5D, etc**)

Standard Serves

Alongside each food there is a "standard serve" size. The "standard" serve is not necessarily a "normal" serve, it is simply there to help us measure food intake. If your child usually eats more or less than the standard serve size for a particular food, please indicate on the **COMMENTS** line what amount is usually eaten.

For example, if when your child eats icecream he/she has one "scoop" instead of our "standard" serve of two "scoops", indicate how often icecream is eaten, and then write "one scoop only" on the comments line.

On the opposite page you will see some examples of how to fill out the questionnaire. Please read these carefully before you start to fill out the answers for your child's diet.

HOW TO ANSWER

| | | | | |
|----------|----------|---|---|---|
| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W | ¹ ₂ ³ D |
| | | | and so on | and so on |

HERE ARE SOME EXAMPLES

| | <u>STANDARD SERVE</u> | | <u>COMMENTS</u> |
|-------------------|---------------------------------|-------|-----------------|
| Custard | 1/2 cup | | _____ |
| Boiled egg | 1 egg | | _____ |
| Cucumber | 3 slices (each 0.5 cm thick) | | _____ |
| Tea | 1 cup | | _____ |
| Beetroot - canned | 2 slices | | _____ |

The child above has, on average :-

- A **standard** serve of custard **three times a week**
 - **Two** boiled eggs **three times a month**
 - Rarely eats cucumber
 - Four cups of tea **every day**
 - **Half a standard** serve (1 slice) of beetroot - canned,
twice a month
-

We realise that some parents have an exact idea of how often their child eats particular foods, whilst others only have an approximate idea. Be as accurate as you can but do not spend too much time choosing your answers.

PLEASE GIVE AN ANSWER FOR EVERY FOOD

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ / ₂ / ₃ M | ¹ / ₂ / ₃ W and so on | ¹ / ₂ / ₃ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| CEREALS | | COMMENTS | |
|---|----------------|----------|-------|
| Porridge/Oatmeal | 1 cup (cooked) | | _____ |
| Muesli | 1/2 cup | | _____ |
| Other breakfast cereal | 1 cup | | _____ |
| Plain bran (raw) | 1 tablespoon | | _____ |
| Wheatgerm | 1 tablespoon | | _____ |
| Bread roll (NOT hamburger buns) | 1 roll | | _____ |
| Fried rice | 1 cup (cooked) | | _____ |
| Boiled rice | 1 cup (cooked) | | _____ |
| Instant noodles (Maggi etc.) | 1 cup (cooked) | | _____ |
| Other pasta (spaghetti, macaroni etc.) | 1 cup (cooked) | | _____ |

Q-1 How many slices of bread does your child **usually** eat? Remember the bread in toast and sandwiches. If bread is not eaten at all, write 'none'.

_____ slices/day OR _____ slices/week ...

Q-2 What type of bread does your child **usually** eat? (Circle the number beside one answer)

- 1 Wholemeal or mixed grain
- 2 White
- 3 About half the time wholemeal and half white
- 4 Other breads (e.g. rye, Hi-Fibe)
(please specify type)
- 5 My child does not eat bread

Q-3 Does your child eat **low-salt** types of bread? (Circle one answer)

ALL or MOST OF THE TIME OCCASIONALLY RARELY/NEVER

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Q-4 Which of the following does your child usually spread on bread or crackers? (Circle one answer)

- 1 Butter
- 2 Polyunsaturated margarine : please name
- 3 Table or cooking margarine; please name
- 4 Reduced-fat margarine (e.g. Era, Becel Light) please name
- 5 Dripping/Lard
- 6 My child does not use anything
- 7 My child does not eat bread or crackers
- 8 Something else : please name

Q-5 For whatever spread your child has, is it **usually** the regular variety or reduced salt? (Circle **one** answer)

- 1 Usually has the regular variety
- 2 Usually has the reduced-salt variety

Q-6 What types of breakfast cereals does your child most commonly eat?

Please name :

Q-7 If your child eats **muesli** is it : (Circle **one** answer)

- 1 Home made muesli
- 2 Pre-packaged muesli

Q-8 How many cups of milk does your child **usually** add to breakfast cereal, porridge or muesli ? (Circle the number closest to the amount he/she has)

- 1 None
- 2 About a half a cup
- 3 About one cup
- 4 About one and a half cups
- 5 About two cups or more (please state how much

Q-9 What type of milk does your child **usually** add to cereals, porridge or muesli? (e.g. whole milk, Hi-Lo, Lite, skim, powdered skim, Shape, Farmers Best, goat's milk, condensed/evaporated milk etc.)?

Type of milk added :

Q-10 How many teaspoons of sugar or honey does your child **usually** add to cereal, porridge or muesli. (Note : 1 level dessertspoon = 2 level teaspoons)

Write the number of level teaspoons your child has here :

Q-11 Does your child **add** salt to porridge? (Circle **one** number)

- 1 Yes
- 2 No
- 3 He/she doesn't eat porridge

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>CEREAL FOODS</u> | | <u>COMMENTS</u> | |
|---------------------------------|---------------------------------------|-----------------|-------|
| Crumpet or Muffin | 1 | | _____ |
| Croissant | 1 | | _____ |
| Fruit Loaf/Currant bread | 1 slice | | _____ |
| Sweet bun/doughnut | 1 | | _____ |
| Crispbread/Cracker | 2 | | _____ |
| Salted biscuits | 3 | | _____ |
| Plain sweet biscuits | 2 | | _____ |
| Fancy biscuits (eg choc-coated) | 2 | | _____ |
| Cake | 1 small cake or 1 slice large cake | | _____ |
| Milk pudding (eg rice, sago) | 1/2 cup | | _____ |
| Steamed sponge - suet | 1/4 small pudding | | _____ |

Q-1 Does your child have milk :

(Circle one for each)

| | | | |
|-----------------------|-----|----|----------------------------------|
| in tea? | YES | NO | DOES NOT DRINK TEA |
| in coffee? | YES | NO | DOES NOT DRINK COFFEE |
| in coffee substitute? | YES | NO | DOES NOT DRINK COFFEE SUBSTITUTE |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|--|--|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W | ¹ ₂ ³ D |
| | | | and so on | and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY HAVE THESE DRINKS?

| <u>BEVERAGES</u> | | <u>COMMENTS</u> | |
|--|--------------------------|-----------------|-------|
| Sustagen (made with powder) | 1 cup | | _____ |
| Sustagen Gold | small carton (300 ml) | | _____ |
| Carton of other flavoured milk (eg chocolate, strawberry etc) | small carton (300 ml) | | _____ |
| Cocoa | 1 cup | | _____ |
| Drinking Chocolate/Milo/ Quik etc. | 1 cup | | _____ |
| Akta-Vite | 1 cup | | _____ |
| Glass of milk (as such) | 1 glass | | _____ |
| Milk shake/Thick shake | regular size | | _____ |
| Tea | 1 cup | | _____ |
| Herbal tea | 1 cup | | _____ |
| Instant coffee | 1 cup | | _____ |
| Ground coffee (eg filter/drip) | 1 cup | | _____ |
| Decaffeinated coffee | 1 cup | | _____ |
| Coffee substitute (eg Caro) | 1 cup | | _____ |

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Q-2 Does your child have cocoa/chocolate/Milo/Akta-Vite with : (Circle **one** number)

- 1 Mostly milk?
- 2 Mostly water?
- 3 About half and half?
- 4 He/she does not drink these drinks.

Q-3 What type of milk does your child **usually** add to tea/coffee/cocoa/chocolate etc?
(Please state the type of milk used eg whole milk, Lite, Hi-Lo, skim, powdered skim, Shape, Farmers Best, goats milk, condensed milk, evaporated milk etc.)

Type of milk added

Q-4 How many **teaspoons** of sugar/honey does your child **usually** have in each cup of :

(Circle **one** number for each drink)

| | | | | | | | |
|----------------------|---|---|---|---|---|---|---|
| Tea? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Coffee? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Coffee substitute? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Cocoa? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
| Milo/Quik/Chocolate? | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

HOW TO ANSWER

| NEVER N | RARELY R | Times a MONTH ¹ ₂ ³ M | Times a WEEK ¹ ₂ ³ W and so on | Times a DAY ¹ ₂ ³ D and so on |
|-------------------|--------------------|---|---|--|
|-------------------|--------------------|---|---|--|

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>DAIRY PRODUCTS and EGGS</u> | | | <u>COMMENTS</u> | |
|--|------------------------|--------|-----------------|-------|
| Cheese | 30 grams (1 slice) | | _____ | |
| Low-fat cottage cheese | 100 gm (1/2 carton) | | _____ | |
| Cream | 1 tablespoon | | _____ | |
| Yoghurt | 200 gm (1 carton) | | _____ | |
| Icecream (from a tub) | 2 scoops | SUMMER | | _____ |
| | | WINTER | | _____ |
| Icecream desserts (eg Symphony, Vienetta) | 1 serving | SUMMER | | _____ |
| | | WINTER | | _____ |
| Icecream (on a stick/cone) | 1 icecream | SUMMER | | _____ |
| | | WINTER | | _____ |
| Vitari | 1 cone | SUMMER | | _____ |
| | | WINTER | | _____ |
| Ice block/Icy Pole | 1 | SUMMER | | _____ |
| | | WINTER | | _____ |
| Custard | 1/2 cup | | _____ | |
| Fried egg | 1 egg | | _____ | |
| Boiled egg | 1 egg | | _____ | |
| Omelette/Scrambled eggs | 2 eggs | | _____ | |

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Q-1 When your child eats cheese, does he/she have the **reduced-salt** varieties (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Rarely or never
- 4 He/she does not eat cheese

Q-2 When your child eats cheese, does he/she have the **reduced-fat** varieties (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Rarely or never
- 4 He/she does not eat cheese

Q-3 When your child eats yoghurt which type is it? (Circle **one** number)

- 1 Plain (eg not fat-reduced)
- 2 Plain, low fat
- 3 Fruit flavoured (not fat-reduced)
- 4 Fruit flavoured, low-fat
- 5 Frozen yoghurt
- 6 He/she does not eat yoghurt

Q-4 When your child eats ice-cream, diet-ice or similar is it **usually**? (Circle **one** number)

- 1 Low calorie
- 2 Regular icecream
- 3 Other (please state)

70

.....

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>MEATS</u> | | <u>COMMENTS</u> |
|---|---|-----------------|
| Steak (eaten as such) | 1 medium (100g) (approximately size of fillet) | _____ |
| Pork chop | 1 medium chop | _____ |
| Lamb chop (loin chop size) | 2 chops | _____ |
| Roast pork/pork fillet | 2 slices | _____ |
| Roast beef/veal | 2 slices | _____ |
| Roast lamb | 2 slices | _____ |
| Sausages | 2 thick or 3 thin | _____ |
| Frankfurters/Saveloys | 2 thick or 3 thin | _____ |
| Bacon | 2 rashers | _____ |
| Ham | 3 thin or 2 thick slices | _____ |
| Luncheon meat/Fritz/ Devon/Windsor etc. | 3 slices (1 cm thick if small nob) | _____ |
| Continental Sausage (salami/Mettwurst etc) | 3 slices | _____ |
| Pate/liver paste | 1 tablespoon | _____ |
| Liver | 1/2 liver (150 gm) | _____ |
| Kidney | 2 kidneys | _____ |
| Brains | 1/2 cup | _____ |
| Pureed meat dishes (canned/bottled) | 1/2 cup | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>MIXED DISHES</u> | | | <u>COMMENTS</u> |
|---|---|-------|-----------------|
| Hamburger WITH bun | 1 medium | | _____ |
| Hamburger patty WITHOUT bun | 1 medium | | _____ |
| Pizza (frozen) | 1 mini or 1/4 large | | _____ |
| Pizza (homemade or take-away) | 1/2 small or 1/4 large | | _____ |
| Sausage roll | 1 large or 2 small | | _____ |
| Meat pie | 1 individual | | _____ |
| Meat pie (homemade) | 1 individual or 1 slice of large pie | | _____ |
| Pastie | 1 individual | | _____ |
| Crumbed veal (schnitzel) | 1 large piece | | _____ |
| Stew/casserole/curry/goulash (with meat or chicken) | 1 cup | | _____ |
| Stew/casserole/curry/goulash (without meat or chicken) | 1 cup | | _____ |
| Chinese meat and veg dish | 1 cup | | _____ |
| Savoury pies/pastries (eg quiche) | 1 individual OR 1 slice of large pie | | _____ |
| Mince meat (eaten as such) | 1 cup | | _____ |
| Mince meat dishes (eg Shepherds' pie) | 1 piece (8x8x4cm) | | _____ |
| Spicy mince added to pastas (eg spag. sauce) | 1/2 cup mince | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>CHICKEN, FISH AND SEAFOOD</u> | | <u>COMMENTS</u> |
|---|---|-----------------|
| Roast/Barbecue chicken | 2 slices of breast or 1 drumstick or 2 wings | |
| Boiled chicken | as above | |
| Crumbed, fried chicken | 4 small pieces | |
| Chicken nuggets | 6 nuggets | |
| Fish - fried (state what type of fish) | 1 piece or 6 nuggets | |
| Fish without batter (steamed, grilled/boiled) (please state what type of fish) | 1 piece | |
| Canned fish (tuna, salmon etc) | 1/3 cup | |
| Fish Fingers | 3 - 4 fingers | |
| Seafood (prawns, crab, lobster etc) | 1/2 cup | |
| Mornay dishes | 1 cup | |

Q-1 If your child eats the following meats, how are they **usually** cooked? (Circle **one** for each food)

| | | | | |
|----------|-------|---------------|------------|-----------|
| Steak | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Chops | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Sausages | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |
| Bacon | FRIED | GRILLED/BAKED | MICROWAVED | DON'T EAT |

Q-2 When your child eats meat with fat on it, does he/she eat : (Circle **one** number)

- 1 All of the fat
- 2 Most of the fat
- 3 About half of the fat
- 4 Little or none of the fat
- 5 He/she does not eat meat

88EOL

Q-3 Does your child have the skin removed from their chicken? (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes (about half the time or less)
- 3 Rarely (less than a quarter of the time)
- 4 Never
- 5 He/she does not eat chicken

Q-4 If your child eats fried fish, in which of the following is it **usually** coated? (Circle **one** number)

- 1 Batter
- 2 Breadcrumbs
- 3 Flour
- 4 Other coating; please name.....
- 5 Fried without coating

Q-5 When your child eats fish coated in batter, crumbs etc how often is it : (Circle **one for each**)

| | | | | |
|---------------------------------------|--------|-----------|--------|-------|
| Coated at home | ALWAYS | SOMETIMES | RARELY | NEVER |
| Pre-packed, frozen cooked at home | ALWAYS | SOMETIMES | RARELY | NEVER |
| Bought ready cooked from fish shop | ALWAYS | SOMETIMES | RARELY | NEVER |

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Q-6 If you buy fresh fish for your child, what variety is it usually?

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ / ₂ / ₃ M | ¹ / ₂ / ₃ W and so on | ¹ / ₂ / ₃ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>CANNED and DRIED VEGETABLES</u> | | | <u>COMMENTS</u> |
|--|------------------|-------|-----------------|
| Potato - canned | 2-3 small | | _____ |
| Potato - packet (powdered) | 1/3 cup (cooked) | | _____ |
| Potato salad | 1/3 cup | | _____ |
| Carrots - canned | 1/3 cup | | _____ |
| Beetroot - canned | 2 slices | | _____ |
| Green beans - canned | 1/3 cup | | _____ |
| Haricot, Lima beans - canned | 1/3 cup | | _____ |
| Baked beans in tomato sauce | 1/3 cup | | _____ |
| Green peas - canned | 1/3 cup | | _____ |
| Lentils - dried/canned | 1/3 cup | | _____ |
| Zucchini salad | 1/3 cup | | _____ |
| Sweetcorn - canned (including creamed corn) | 1/3 cup | | _____ |
| Mushrooms - canned | 6-7 small ones | | _____ |
| Mushrooms - canned in sauce | 1/3 cup | | _____ |
| Olives | 3 medium | | _____ |
| Gherkins/Pickled onions | 3 pieces | | _____ |
| Pureed vegetables (canned/bottled) | 1/3 cup | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

The following list of foods contains some vegetables that may be eaten much more frequently at some times of the year than others (eg in the warmer or cooler weather). Please fill in how often each food is eaten in **BOTH** the warmer months of the year (**SUMMER**) and the cooler months (**WINTER**).

For example :- If your child usually has :

A standard serve of peas about **twice a week** during the **warmer** months of the year and about **every day** during the **cooler** months :

and:

Two medium potatoes (roasted) a **week** throughout the **year** :

You would write :

| | | <u>Summer</u> | <u>Winter</u> | |
|------------------|----------|---------------|---------------|-------|
| Green peas | 1 cup | | | _____ |
| Potato - roasted | 1 medium | | | _____ |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>SEASONAL VEGETABLES</u> | | <u>Summer</u> | <u>Winter</u> | |
|-------------------------------------|-------------|---------------|---------------|-------|
| Potato - fresh & mashed (with milk) | 1/3 cup | | | _____ |
| Potato - fresh, boiled | 1 medium | | | _____ |
| Potato - roasted | 1 medium | | | _____ |
| French fries/hot chips | 17-18 chips | | | _____ |
| Potato Gems/ Pommes Noisettes | about 5 | | | _____ |
| Carrots (fresh/frozen) | 1/3 cup | | | _____ |
| Turnip/Swede (fresh/frozen) | 1/3 cup | | | _____ |
| Broad beans (fresh/frozen) | 1/2 cup | | | _____ |
| Green beans (fresh/frozen) | 1/3 cup | | | _____ |

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| <u>SEASONAL VEGETABLES (continued)</u> | | <u>Summer</u> | <u>Winter</u> |
|--|---------------------------------|---------------|---------------|
| Green peas (fresh/frozen) | 1/3 cup | | |
| Cabbage | 1/3 cup | | |
| Brussels sprouts (fresh/frozen) | 5 - 6 | | |
| Silver beet/spinach (fresh/frozen) | 1/3 cup | | |
| Broccoli (fresh/frozen) | 1/3 cup | | |
| Cauliflower (fresh/frozen) | 1/2 cup | | |
| Pumpkin | 1/3 cup | | |
| Sweetcorn (fresh/frozen) | 1 small cob | | |
| Zucchini (courgettes) | 1 medium sized | | |
| Onion - fried | 1/4 cup | | |
| Onion (raw, baked, boiled) (fresh/frozen) | 1 medium | | |
| Tomato - fresh | 1 medium | | |
| Tomato - grilled/fried | 1/2 medium | | |
| Lettuce | 2 small leaves | | |
| Cucumber | 3 slices (each 0.5 cm thick) | | |
| Coleslaw | 1/2 cup | | |
| Celery (fresh/frozen) | 1 x 15cm stick | | |
| Capsicum (Green pepper) (fresh/frozen) | 2 strips (each 0.5 cm thick) | | |
| Mushrooms - fresh | 6-7 small ones | | |
| Sprouted bean shoots | 1/3 cup | | |
| Fried mixed vegetables (eg stir fried) | 1/2 cup | | |

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Q-1 When you use canned vegetables, are they **reduced-salt** varieties? (Circle **one** number)

- 1 Always or nearly always
- 2 Sometimes
- 3 Never or rarely
- 4 Only for some vegetables (please state which.....)

Q-2 Is salt added to the cooking water when boiling the following foods? (Circle **one** for each food)

| | | | |
|----------------|---------|-----------|-------|
| Vegetables | USUALLY | SOMETIMES | NEVER |
| Pasta and rice | USUALLY | SOMETIMES | NEVER |

Q-3 If salt is added to the cooking water when boiling foods, is the water : (Circle **one** number)

- 1 Lightly salted
- 2 Medium salted
- 3 Heavily salted
- 4 Salting is highly varied
- 5 Salt is not added to cooking water

Q-4 How often do you add salt to your child's meals **after** they are cooked? (Circle **one** number)

- 1 Rarely or never
- 2 Sometimes
- 3 Always or nearly always

Q-5 When you add salt at the table to your child's meals, how much is **usually** added?
(Circle **one** number)

- 1 A light sprinkle
- 2 A medium sprinkle
- 3 A heavy sprinkle
- 4 Salting is highly varied
- 5 Salt is not added at the table

Q-6 When you cook vegetables for your child which of the following methods is the one **most** commonly used? (Circle **one** number)

- 1 Boiled in a little water
- 2 Boiled in a lot of water
- 3 Steamed
- 4 Cooked in a pressure cooker
- 5 Microwaved
- 6 Stir-fried

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- Q-7
- | | | | |
|---|--|---|----------------------------|
| 1 | Vegetable oils (olive, sunflower etc.) | 4 | Dripping/lard/ meat juices |
| 2 | Cooking or table margarine | 5 | Polyunsaturated margarine |
| 3 | Butter | 6 | Nothing |

From the list above write which type of fat/oil is most commonly used :

- (a) When roasting/frying meats/fish.....
- (b) When roasting/frying vegetables.....
- (c) On vegetables when served (eg butter on peas).....

Q-8 Is butter or margarine added to your child's potatoes when they are mashed?
(Circle one number)

- 1 Yes, always
- 2 Yes, occasionally
- 3 Never

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|----------|----------|---|---|---|
| N | R | ¹ ₂ ₃ M | ¹ ₂ ₃ W | ¹ ₂ ₃ D |
| | | | and so on | and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>FRUIT</u> | | <u>COMMENTS</u> | |
|---|---------------------------------|-----------------|-------|
| Orange, Mandarin, Grapefruit | 1 medium | | |
| Apple, Pear - fresh/baked | 1 medium | | |
| Banana | 1 medium | | |
| Fresh fruit salad | 1 cup | | |
| Dried fruit (apple/apricot etc) | 4-5 pieces | | |
| Raisins, sultanas or currants | 1/3 cup | | |
| Fruit in syrup or stewed (including fruit salads) | 1/2 cup | | |
| Fruit canned in water (low-cal) (including fruit salads) | 1/2 cup | | |
| Fruit pie or pastry or fritters | 1 small pie or 1 slice large | | |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

The fruits listed below are only available for a short time during the year. Therefore we only want you to record how often your child has them when they are IN SEASON.

HOW OFTEN DOES YOUR CHILD EAT THESE FOODS WHEN THEY ARE IN SEASON?

| <u>SEASONAL FRUITS</u> | | <u>COMMENTS</u> | |
|------------------------|---------------|-----------------|-------|
| Berries - fresh/frozen | 3/4 cup | | _____ |
| Melon (not watermelon) | 1 large slice | | _____ |
| Peach - fresh | 1 medium | | _____ |
| Plum - fresh | 3-4 plums | | _____ |
| Nectarine - fresh | 1 medium | | _____ |
| Apricot - fresh | 3 apricots | | _____ |
| Grapes - fresh | about 20 | | _____ |
| Pineapple - fresh | 1 slice | | _____ |
| Avocado | 1/2 a medium | | _____ |

Please list here, along with your child's standard serve size, any other fruit that your child eats (eg mango; pureed, canned/bottled fruits)

_____
 _____

HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>NUTS and SNACKS</u> | | <u>COMMENTS</u> | |
|--|----------------------------------|-----------------|-------|
| Potato crisps, Twisties etc | 1 small bag or 14 - 15 pieces | | _____ |
| Peanuts (fresh) | 9 - 10 nuts | | _____ |
| Nuts - salted & cooked | 9 - 10 nuts | | _____ |
| Other unsalted nuts (fresh walnuts/almonds etc) | 5 - 6 nuts | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ₃ M | ¹ ₂ ₃ W and so on | ¹ ₂ ₃ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>SOUPS</u> | | <u>COMMENTS</u> | |
|-------------------------------|-------|-----------------|-------|
| Canned soup (eaten as such) | 1 cup | WINTER | _____ |
| | | SUMMER | _____ |
| Packet soup (eaten as such) | 1 cup | WINTER | _____ |
| | | SUMMER | _____ |
| Homemade soup (eaten as such) | 1 cup | WINTER | _____ |
| | | SUMMER | _____ |

Write an example of the type of soup your child most often eats (eg canned tomato; homemade pea and ham)

.....

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY EAT THESE FOODS?

| <u>CONFECTIONERY, JAMS AND SAUCES</u> | | | <u>COMMENTS</u> |
|---|---------------------------|-------|-----------------|
| Chocolate | 1 small bar (50 grams) | | _____ |
| Chocolate covered bar (eg Mars/Bounty) | 1 bar | | _____ |
| Individually wrapped lollies, eg toffees | 4 - 5 lollies | | _____ |
| Packet lollies (eg Lifesavers/Polos) | 1 small packet | | _____ |
| Muesli bar/Health bar | 1 bar | | _____ |
| Honey, jam, marmalade | 1 tablespoon | | _____ |
| Vegemite, marmite etc | 1/2 teaspoon | | _____ |
| Thick sauces (tomato/HP etc) | 1tablespoon | | _____ |
| Polyunsaturated Mayonnaise/ Salad cream | 1 tablespoon | | _____ |
| Regular Mayonnaise/ Salad cream | 1 tablespoon | | _____ |
| Low calorie salad dressings | 1 tablespoon | | _____ |
| Polyunsaturated salad dressings | 1 tablespoon | | _____ |

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HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

ABOUT HOW OFTEN DOES YOUR CHILD USUALLY HAVE THESE FOODS?

| <u>BEVERAGES</u> | | <u>COMMENTS</u> |
|---|---------------------|-----------------|
| Glass of cordial | medium glass | _____ |
| Glass of cola (eg Coca Cola) | medium glass | _____ |
| Glass of fizzy drink Includes mineral water with juice | medium glass | _____ |
| Glass of low-calorie fizzy drink | medium glass | _____ |
| Fruit drink (eg Fruit Box) | 250 ml carton | _____ |
| Pure fruit juice | medium glass | _____ |
| Vegetable juice | small glass | _____ |
| Water/Spring water | medium glass | _____ |
| Mineral Water | medium glass | _____ |

OTHER FOODS COMMENTS
PLEASE LIST ANY OTHER FOODS HERE AND ON THE NEXT PAGE THAT YOUR CHILD
HAS EATEN, PARTICULARLY TYPES OF WHOLE GRAINS, NUTS, SEEDS AND FISH

| | | |
|--|--|--|
| | | |
| | | |
| | | |
| | | |
| | | |

If your child has any other foods or drinks that we have not mentioned, at least once a month, please write them down here and tell us how often he/she has them, using the same response scale as before (eg 1D, 3M etc).

HOW TO ANSWER

| NEVER | RARELY | Times a MONTH | Times a WEEK | Times a DAY |
|-------|--------|--|---|---|
| N | R | ¹ ₂ ³ M | ¹ ₂ ³ W and so on | ¹ ₂ ³ D and so on |

FOODS AND DRINKS MY CHILD CONSUMES THAT HAVE NOT BEEN MENTIONED :

| Name of Food | His/her usual serve size | How often is it eaten? |
|--------------|-----------------------------|---------------------------|
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |
| _____ | _____ | |

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VITAMIN AND MINERAL SUPPLEMENTS

If your child takes any vitamins or minerals, or any other dietary supplements, such as fibre tablets, lecithin, kelp, yeast etc, please fill in the table below. (Check the label on the box or bottle if you are unsure of some of the answers).

| BRAND (eg Nyal) | NAME OF PRODUCT (eg vitamin C pill) | SIZE OF DOSE (eg 250 mg) | NUMBER OF DOSES (eg 2 per day) |
|--------------------|--|-----------------------------|-----------------------------------|
| _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ |

WHAT WAS YOUR CHILD'S WEIGHT AND LENGTH AT BIRTH

WAS YOUR CHILD BREAST FED? (Please circle answer) YES NO

IF SO, FOR HOW LONG WAS YOUR CHILD BREAST FED?

HAS YOUR CHILD CHANGED HIS/HER DIET IN THE PAST 2 - 3 MONTHS? YES NO

IF SO, WHAT ARE THESE CHANGES?

.....

THANK YOU VERY MUCH FOR YOUR PARTICIPATION IN THIS STUDY

REMEMBER

PLEASE PUT THIS QUESTIONNAIRE INTO THE

REPLY PAID ENVELOPE PROVIDED

AND RETURN IT TO US

AS SOON AS POSSIBLE

We would welcome any comments you may have regarding
this questionnaire below

Appendix 6 - Consents

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

The Raine Study – physical activity levels, respiratory disease, and stress responsiveness in early adolescence

Adolescent consent form

I, _____ have read the Adolescent Information Sheet explaining the 13 year old follow up. Any questions I have asked have been answered so that I understand what is going to happen.

I understand that I don't have to participate if I don't want to.

I agree that the research data gathered from the results of this study may be published, provided that my name is not used.

I agree to participate in the following parts of the study (please circle as appropriate):

- | | | |
|---|-----|----|
| - Child questionnaire | yes | no |
| - Physical examination, measurement, blood pressure, physical fitness test, coordination test | yes | no |
| - School survey – questionnaires for the school principal and classroom teacher | yes | no |
| - Pedometer test, 7 day physical activity diary, 24 hour blood pressure test | yes | no |
| - Blood & urine test (Genetic test – see separate form) | yes | no |
| - Stress test | yes | no |
| - Test of bronchial responsiveness and lung function test | yes | no |
| - Skin prick tests for allergies | yes | no |

Dated _____ day of _____ 20 _____

Signed _____

I, _____ have explained the above study to the signatory who states that he/she understand the same.

Signed _____ (Investigator)

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

**The Raine Study – physical activity levels, respiratory disease,
and stress responsiveness in early adolescence**

Parent consent form (adolescent assessment)

I, _____ have read the Parent Information Sheet explaining the 13 year old follow up. Any questions asked have been answered to my satisfaction.

Withdrawal from the study at any stage will be possible and will not interfere with access to routine care.

I agree that the research data gathered from the results of this study may be published, provided that names are not used.

I agree to my son/daughter _____ participating in the following parts of the study (please circle as appropriate):

- | | | |
|---|-----|----|
| - Child questionnaire | yes | no |
| - Physical examination, measurement, blood pressure, physical fitness test, coordination test | yes | no |
| - School survey – questionnaires for the school principal and classroom teacher | yes | no |
| - Pedometer test, 7 day physical activity diary, 24 hour blood pressure test | yes | no |
| - Blood & urine test (Genetic test – see separate form) | yes | no |
| - Stress test | yes | no |
| - Test of bronchial responsiveness and lung function test | yes | no |
| - Skin prick tests for allergies | yes | no |

Dated _____ day of _____ 20 _____

Signed _____ (Parent/Guardian)

I, _____ have explained the above study to the signatory who states that he/she understand the same.

Signed _____ (Investigator)

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

**The Raine Study – physical activity levels, respiratory disease,
and stress responsiveness in early adolescence**

Parent consent form (parent assessment)

I, _____ have read the Parent Information Sheet explaining the 13 year old follow up. Any questions asked have been answered to my satisfaction.

Withdrawal from the study at any stage will be possible and will not interfere with access to routine care.

I agree that the research data gathered from the results of this study may be published, provided that names are not used.

I agree to participate in the following parts of the study (please circle as appropriate):

- | | | |
|--|-----|----|
| - Height, weight and blood pressure measurement | yes | no |
| - Blood test (Genetic test – see separate form) | yes | no |

Dated _____ day of _____ 20 _____

Signed _____

I, _____ have explained the above study to the signatory who states that he/she understand the same.

Signed _____ (Investigator)

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

**The Raine Study – physical activity levels, respiratory disease,
and stress responsiveness in early adolescence**

Parent consent form: adolescent genetic studies

I consent to the collection of blood (via venepuncture) from my child from which DNA will be extracted and stored for the gene studies that have been explained to me as part of The Raine Study – 'physical activity levels, respiratory disease, and hypothalamo-pituitary-adrenal responsiveness in early adolescence'.

I consent to my child's DNA being used for gene research into the development of asthma, allergies, blood pressure variability, and sugar and fat metabolism.

I understand that the DNA will not be used for purposes other than that specified above and will not be used for diagnostic purposes.

Name of child: _____

Name of Parent / Guardian: _____

(Parent / Guardian)

Date

Investigators statement

I _____ have carefully explained to the parent / guardian the nature of the above project. I hereby certify that to the best of my knowledge, the person who is signing this consent form understands clearly the nature, demands, benefits and risks involved in his / her participation and his/her signature is legally valid. A medical problem or language or education barrier has not precluded this understanding.

Signature of Principal Investigator or Proxy

Date

| | | | | |
|--|--|--|--|--|
| | | | | |
|--|--|--|--|--|

**The Raine Study – physical activity levels, respiratory disease,
and stress responsiveness in early adolescence**

Parent consent form: parent genetic studies

I consent to the collection of blood (via venepuncture) from me, from which DNA will be extracted and stored for the gene studies that have been explained to me as part of the Raine Study – 'physical activity levels, respiratory disease, and stress responsiveness in early adolescence'.

I consent to my DNA being used for gene research into the development of blood pressure variability, and sugar and fat metabolism.

I understand that the DNA will not be used for purposes other than that specified above and will not be used for diagnostic purposes.

Name: _____

Signature

Date

Investigators statement

I _____ have carefully explained to the signatory the nature of the above project. I hereby certify that to the best of my knowledge, the person who is signing this consent form understands clearly the nature, demands, benefits and risks involved in his/her participation and his/her signature is legally valid. A medical problem or language or education barrier has not precluded this understanding.

Signature of Principal Investigator or Proxy

Date

Appendix 7 – Child Information Sheet

2 – Split assessment

The Raine Study – physical activity levels, respiratory disease, and stress responsiveness in early adolescence

Adolescent information sheet

Dear

It is time for the 13 year follow-up of Raine Study kids and we want to see YOU! We want to check out how much physical activity you do, see if you have asthma, and see if you are allergic to anything. We've got a lot of different tests and things for you to do, and your OWN questionnaire. There are three parts to the assessment. Here's how it goes.

We'd like you to visit us at the Institute for the first part of your assessment. We won't be doing anything without your Mum's or Dad's permission. This part of the assessment should be really fun ... we're going to do a stress test and check out your breathing. With the stress test we get you to suck in some gas that takes your breath away for a moment and we measure cortisol that's in your saliva. All we have to do is put a cotton bud in your mouth a few times. After that, we do some special breathing tests and some skin allergy tests. Most of you guys had these tests when you were just 5 years old. It shouldn't take too long. Before you leave, we're going to fit you out with a pedometer (a little device that measures every step you take) and a laptop computer (if you don't have a computer at home) with a built in activity diary program. Some lucky kids will even get to wear a special little blood pressure monitor for 24 hours! Finally, we're going to give you a diary to take home and some measuring cups and spoons for you to record everything you eat and drink for the next three days. When you have finished it's just a matter of popping the diary in the envelope we give you and posting it back to us.

We want you to wear the pedometer and do the physical activity diary on the computer for 7 days. You'll be able to contact us at the Raine office if our technology gets too hard to handle and you need some help. After a few days we'll visit you early in the morning to see how things are going. We also want to do a blood test and collect some urine. We can put that special cream on that numbs your skin if you want it. We want to get some blood from your Mum and Dad too. If you don't want to, we'll just leave it out ... you don't have to do anything you don't want to.

After 8 days, we'll get your parents to bring you back to the Institute. With a bit of luck, you'll remember to bring our equipment in with you. We hope so, because it costs a lot of money and we can't afford to lose it! We'd like you to do a questionnaire on a laptop computer - this should take about an hour with some help from us. Then, after checking your height and weight and blood pressure we're going to give you a fitness test - things like sit-ups, basketball throws and a ride on an exercycle. If you're still able to move after that we're going to test your coordination! Do you remember the finger tapping test, broad jump, and the grip strength stuff when you were 10? Well,

2 – Split assessment

we're going to do those ones again ... we've even got a physical education expert to help us! Last but not least we want to take your photo to assess your sitting posture.

All in all, there's a lot to it. The information we get is going to be really useful to help us to know more about things like asthma and allergies and physical fitness and that sort of thing. You can be sure that all the information we have about you and your family is confidential. That means that we won't tell anyone about your results. When we do our analysis we focus on results for large groups, NOT individuals!

By the way, if you tell us about something you don't want your Mum and Dad to know, it's not a problem. The things you tell us are confidential. And, we're here to HELP! Whether you decide you want to talk with your Mum and Dad about it or not, we'll make sure you get things sorted out.

We look forward to seeing you again soon.

Best wishes
The Raine Study Team

Phone: (08) 9489 7793, 9489 7794, 9489 7796

Appendix 8 – Parent Information Sheet

1 – Full day assessment

The Raine Study – physical activity levels, respiratory disease, and stress responsiveness in early adolescence

Parent information sheet

Dear Parent/Caregiver

As part of the 13 year-old follow-up we plan to assess the children's levels of physical activity and their respiratory status. We asked a number of questions about physical activity when your child was 10 years old. We would now like to assess your child's level of physical activity in a comprehensive manner and relate this information to their level of physical fitness and coordination. We would like to know if daily activities such as walking to school and doing chores around the home contribute significantly to fitness and health. You may remember the respiratory status assessments that took place when your child was about 6 years old. The tests we plan this time are similar but will give us a lot more information as the children are now able to perform more complex tasks. We want to investigate factors that relate to asthma and allergies, particularly to the type of asthma that is likely to last into adult life.

We would like to carry out a three-stage assessment. **Stage one** will involve the assessment of your child at the Telethon Institute for Child Health Research and the Clinical Research Centre, Princess Margaret Hospital. We will be asking you and your child's secondary caregiver (where applicable) to complete separate questionnaires. The questions are similar to those we have asked in previous follow-ups. Parents will be asked to return completed questionnaires at this time. While you are at the institute we will also ask you to complete a food frequency questionnaire detailing your study child's eating habits and ask you questions about your child's general mood. The adolescent assessment will comprise height, weight, respiratory examination, a stress test, lung function test, bronchial responsiveness test, and skin prick test for allergies. Attending parents will also be asked to have height, weight and blood pressure measured. It is envisaged that this assessment will take 2.5 hrs. Details of the stress test and the bronchial responsiveness test are presented below.

Stress test

The Hypothalamic-Pituitary-Adrenal (HPA) axis is a vital part of the body and stimulates the body's normal production of steroids. When our body is faced with stress of any type, including emotional or physical stress, the HPA axis stimulates an increase in the amount of steroid (cortisol) our body produces. There is a suggestion that some adults with asthma produce less steroid in response to stress than non-asthmatics. We do not know whether this contributes to the development of the asthma or is a consequence of the asthma. There is no information whether this situation also exists in children. The Raine Study is ideally suited to examine the relationships between the physiological response to stress and asthma in children.

HPA axis responsiveness will be tested using a "single breath test" in which the children will inhale a single vital capacity breath of a gas mixture comprising 65% Oxygen (as apposed to 21% in normal air) and 35% Carbon dioxide (which we breathe out with each breath but which is present in very low concentrations in normal air). Inhaling this gas is not at all harmful, yet it results in the HPA axis stimulating cortisol production. After inhaling the gas mixture, your child will experience a transient (approx 5-10 second) feeling of light-headedness and notice an increased respiratory drive, i.e. their breathing rate increases for a minute or so. We measure the amount of cortisol, in the children's saliva, that is stimulated by the test. We do this by having them hold a cotton swab in their mouth for about one minute each time we want to measure cortisol. There are no blood tests involved. As this is a stress test, it is important that the children are relaxed before the test starts. We will ask them to rest for about 30 minutes in the room where the test is conducted. During this period, and for about 30 minutes after the breath of gas mixture, we will collect a saliva sample approximately once every 5 to 10 minutes, to allow us to measure the cortisol response. We will also store some of the saliva to allow us to analyse it for the presence of antibodies (funding permitting).

1 – Full day assessment

We wish to relate how much cortisol is produced by a stress test to how your child deals with life stresses. One way of measuring your child's response to life stresses is to measure how anxious they are, both at the time of the test and in general. We can do this using a standardized instrument (a questionnaire that your child fills out) called Spielberger's State and Trait Anxiety test.

Bronchial responsiveness testing

During the six year follow-up we were able to measure bronchial responsiveness in some of the children. These tests tell us how sensitive your child's airways are. Children with asthma generally have more sensitive airways, which means that they react more than usual to environmental stimuli. However, everybody's airways are sensitive to some degree, so this is a test we want to perform on children who do not have asthma, as well as on those who do. We have two ways of measuring bronchial responsiveness, either using methacholine – a chemical analogue to a naturally occurring body chemical that tells us how sensitive the airways are or using a bronchodilator, such as Ventolin, which is used as an asthma treatment because it opens narrowed airways.

Methacholine challenge

The test is done by taking a number of breaths of a nebulized solution and measuring lung function 30 and 90 seconds later. We always start the test using a saline solution, followed by increasing doses of methacholine. People with sensitive airways will have some narrowing of their airways with the methacholine and we can see this as a reduction in their lung function. We stop the test if lung function falls by 20%, a level that most people will not even notice. For comparison lung function usually falls by more than 50% during a moderate asthma attack. Some children may experience some coughing or mild wheezing at the end of the test. All children whose lung function has fallen at all will be given a Ventolin inhalation at the end of the test and this will completely reverse any fall in lung function.

Bronchodilator response

Children who are unable to undertake the methacholine challenge will receive bronchodilator inhalation (10 puffs from a large volume aerosol-holding chamber / spacer), as per Department of Respiratory Medicine protocol. Lung function will be repeated 10 minutes post bronchodilator to see if the Ventolin improves their lung function.

We will be asking you for separate permission for each part of the study so that you can feel free to participate in the study without feeling pressured to agree to it all. You may, of course, withdraw your child at any stage without prejudicing your child's right and access to the best medical attention available at Princess Margaret Hospital.

With your permission we will ask your child to complete a questionnaire for us. The questionnaire is mainly about how children perceive themselves, but there are questions about 'risk taking' behaviour, such as smoking and drinking alcohol as well. We have included a copy for you to read. The information contained in this questionnaire is confidential. If your child tells us of a health or social problem he/she does not want you to know about, the matter will be referred to a panel consisting of: two child health nurses; a clinical psychologist; an adolescent paediatrician; and a paediatric social worker. No fewer than three members of this group will determine how to proceed in each case. An appropriate response will be mounted with the knowledge and cooperation of your child.

As part of this study we will be doing a number of tests on you and your child. Some results, such as lung function and allergy tests, we will be able to give you immediately. Other tests will take some time to run in the laboratory; therefore we will send you these results when available. If any of your results are outside the normal range, or we are worried you or your child may have a problem you were not aware of, we will recommend that you see your General Practitioner. However, for some of the tests, such as immunology and genetics, we are not able to send you results, because at this stage in our knowledge we do not know how to interpret what these tests mean for an individual.

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Stage two will commence in the afternoon, following a brief break for lunch. Your child will need approximately 3 hours to complete a questionnaire (administered on laptop computer – paper copy enclosed for your perusal), a physical fitness assessment, and an assessment of coordination. In addition, height/weight, waist girth and blood pressure will be measured. We will also take a series of digital photos of your child to assess their standing and sitting posture. These photos will be altered to so that all identifying features will be masked. The school principal and your child's form teacher will also be asked to complete questionnaires asking about the school environment, your child's participation within that environment and their academic achievement.

Stage three will comprise the period of 7 days following the assessment. At the end of the assessment we will teach your child to use a pedometer (a small instrument that measures the number of steps taken when walking) and a computer based activity diary to monitor their level of activity on a daily basis for 7 days. A small group of children will be asked to use an ambulatory blood pressure monitor for a period of 24 hours. Children and/or parents who have questions about any of this equipment can contact the Raine staff during normal hours on the numbers provided. A phlebotomist/enrolled nurse will visit you at home to take an early morning fasting blood sample and urine from consenting children and their parents. We will need to collect a total of 50 mls of blood from children and 10mls of blood from parents. Just as we have done on previous occasions, local anaesthetic cream (EMLA) will be used for all children and parents who request it.

We will use your child's blood and urine to do a number of studies:

- First, we will measure levels of fats, glucose and insulin;
- Second, we will measure levels of allergic antibodies and markers of inflammation, and the response of the immune system to environmental exposures, especially to infections and allergens;
- Third, we would like to measure the levels of some hormones that are involved in regulating the immune system (eg cortisol, estrogen and testosterone);
- Fourth, we will test for exposure to environmental chemicals and metals, including lead, pesticides, and PCBs (polychlorobiphenols). We can be exposed to these chemicals without our knowledge as they can occur in the air we breathe, the water we drink and in the food we eat. We want to study these chemicals as they all can have effects on the development of the immune system and may influence the risk of asthma and allergies;
- Fifth, we plan to store plasma which will be used to estimate free fatty acid and vitamin levels as an indicator of dietary intake;
- Sixth, we would like to store your child's DNA so that we can study inherited factors associated with asthma, allergies, blood pressure variability, obesity and sugar metabolism.

We will use your blood to measure levels of fats, glucose and insulin, and we would like to store your DNA so that we can study inherited factors associated with blood pressure variability, obesity and sugar metabolism. We have given you more detailed information about these genetic studies on a separate information sheet.

We would like you to feel free to ask any questions you may have about any aspect of the study. It is important that you understand why we are asking you to allow your child to participate in this part of the study.

We would like to assure you that all information we collect is strictly confidential. The Institute (ICHR) is bound by the Privacy Act 1988 and abides by the National Privacy Principles at all times. If you have any concerns or complaints regarding the way this study is being conducted you can contact the Executive Director of Medical Services on (08) 9340 8222.

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Nick Sloan BSc (Hons)
Raine Study Coordinator
Phone: (08) 9489 7795

Professor Peter Sly MD FRACP
Respiratory Physician, Princess Margaret Hospital
Head, Division of Clinical Sciences
Telethon Institute for Child Health Research
Phone: (08) 9340 8222 (24 hours)

Professor Stephen Zubrick PhD
Head, Division of Population Sciences
Telethon Institute for Child Health Research

Appendix 9 - CSIRO Protocol

Protocol for sending dietary booklets to CSIRO in Adelaide for data entry and checking.

Purchase 50 A3 3kg Express Post envelopes from Australia Post on study account. Each envelope will hold up to 3kg or the equivalent of 40 booklets (only), therefore 50 envelopes will handle 2000 booklets.

Collect returned dietary booklets, and record study ID on sheet. When 40 booklets are collected they should be posted by Express Post to Sally Record, at the address below.

Specifically, when 40 booklets have been collected, they should be tied together with string (for safety) and placed in the Express Post envelope. Each envelope registration number sent to CSIRO should be recorded, and the registration sticker saved in a dedicated place. Please put the study name and phone number as the return address on the Express Post envelope as follows:

From: Dr Wendy Oddy/Mr Nick Sloan
Telethon Institute for Child Health Research
(Postal) PO box 855, West Perth, WA 6872 Australia
(Deliveries) Loading Dock Area, 100 Roberts Road
Subiaco 6008
Western Australia

Phone (08) 9489 7879/ 94897795 Fax: (08) 9489 7700

The envelope should then be posted to Sally Record at the address detailed as follows:

To: Sally Record
CSIRO Health Sciences and Nutrition
(Postal:) Box 10041, Adelaide BC, SA 5000 Australia
(Deliveries:) Gate 13, Kintore Ave.,
Adelaide 5000
South Australia

Ph: (08) 8303 8817 Fax: (08) 8303 8899

Sally Record will coordinate the entry of data of the dietary booklets at CSIRO. When data entry has occurred, individual dietary reports will be electronically sent to Dr Oddy and the study coordinator. The reports will be identifiable by study ID only, and are for distribution to study participants. A research nurse dedicated to this task will either email or post each report to study participants.